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CONGENITAL DEFECTS IN THE DIAPHRAGM *

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Defects in the diaphragm with displacement of the abdominal viscera into the thorax have intrigued observers since the days of Paré. Four cases of the massive congenital type recently came to our attention. Together with 5 other cases from the records of 4000 autopsies at the New Haven Hospital they form the subject of this report. Certain possibly important anatomical features, particularly the relations of the serous membranes and vessels and their variations, have received scant attention heretofore. The material at hand, furthermore, affords us the opportunity of analyzing critically previous theories of pathogenesis, of reviewing significant clinical data, and of examining the anatomy with the possibilities of therapeutic approach in mind.

TYPES AND INCIDENCE

Statistics: Congenital hernias are much less frequent than the acquired or traumatic type but form a clinically significant group. Hedblom classified 116 of 600 diaphragmatic hernias as congenital in origin. The group has recently been subdivided by Gruber into three main classes as follows:

1. Those resulting from (*a*) defect in the Anlagen (agenesis), or (*b*) failure of union of the Anlagen. Total failure of development of the diaphragm or of half of the diaphragm is exceedingly rare. The phrenic nerves in such cases are absent on the affected

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sides and there is no trace of a crus. The commonest type of congenital hernia, however, results from failure of union of the Anlagen. This is also referred to as a "false hernia" because no peritoneal sac precedes the ectopic viscera.

2. Those resulting from defects in the united diaphragm; (*a*) failure of deposition of muscle, general or local (true hernias). A type of the latter is the hiatus hernia, *e.g.* parasternal (Fitzmaurice-Kelly); (*b*) diverticulum, that is, a localized outpouching of the definitive muscular or tendinous structure (Glaser).

3. Those displacements of the viscera resulting from dystopia of the diaphragm because of agenesis of the lung. Cases in this group are so rare as to be curiosities (see Schmit).

It is obvious that Types 1 and 3 must have their origin earlier in ontogeny than Type 2.

Large defects are situated in the dorsolateral part of the diaphragm. They are the most frequent of the congenital types and will be the chief subject of this report. They are either of Type 1*b* or 2*a* of Gruber. Of 256 congenital cases reviewed by Liepmann 208 were in the dorsolateral part of the diaphragm, but only 17 were through the sternocostal foramen, 16 in the centrum tendineum and 5 through the esophageal hiatus.

"False" hernias (Gruber, Type 1*b*) are more frequent than "true" (Gruber, Type 2*a*). According to Jahn the latter comprise only 10–12 per cent of all of the massive congenital types, but Cailloud states their incidence to be as high as 1 in 4.

Hernias are said to be more frequent on the left side but authorities differ slightly as to their predominance, 75–85.3 per cent (Cailloud, Hedblom, Jahn, von Gössnitz). Jahn, quoting the data of Thoma, Grosser and Eppinger, states that 31 per cent of true hernias are on the right but that only 19 per cent of false hernias are on this side. Possible reasons for this disparity will be presented when the mechanism of the hernias is discussed. In a long series tabulated from many sources von Gössnitz found males to be more often affected than females (65.4–34.6 per cent) but not in such excess as in the traumatic type.

Technic

To preserve the relations of the large defects a method was employed whereby the membranes and viscera were permanently

preserved without disfiguring the body. The parts were first examined *in situ*. The spinal column was then transected at the eighth thoracic vertebra and again in the upper lumbar region. The entire costal arch together with the lower part of the sternum was left intact, in articulation with the spinal column posteriorly. All of the thoracic and abdominal viscera were then shelled out in relation with the entire serous body wall. Thus the hoop formed by the costal arch and spinal column served to support the hernial orifice, membranes and viscera. Cases 1, 2, 3 and 4 were studied by this method and were permanently preserved in modified Kaiserling fluid. Data in the other cases were obtained from records at the New Haven Hospital.

Case Reports

Case 1. False Hernia *

Clinical History: This male child was born of a healthy mother aged 32 years after a labor of 25¾ hours. The mother had had 1 previous pregnancy, spontaneously terminated in an abortion at 2 months, about 1 year previously. The last pregnancy and labor were uncomplicated.

Valiant attempts to breathe were made by the infant but the lungs could not be expanded even when artificial inflation by means of a tube was attempted.

Clinical Diagnosis: ? tracheal atresia.

Anatomical Diagnoses: Congenital defect in the diaphragm (left); intrathoracic ectopia of the stomach, small intestine, colon and spleen; dextro-displacement of mediastinum; and pulmonary atelectasis involving particularly the left lung.

Gross Examination

Externally this large (3500 gm., 53 cm.) infant shows nothing of note. The abdomen is not distended but is firm to palpation and dull to percussion. When the abdominal incision is made only portions of the liver and large intestine are found within the peritoneal cavity. Palpation and inspection reveal a large opening in the diaphragm through which the remaining viscera have entered the left thoracic cavity. They are not preceded by a hernial sac. The right half of the diaphragm is intact. The posterior wall of the thorax is lined by a mesothelial membrane continuous with that of the peritoneum. The ectopic organs have compressed the bilobed left lung to a completely airless mass of dark red tissue and have displaced the mediastinum entirely to the right of the

* Meriden Hospital, autopsy No. M2.

midline. Only the anterior margins of the three lobes of the right lung are crepitant. There is no evidence of obstruction of the trachea and bronchi and the heart displays no anomalies.

The defect in the diaphragm is 4 cm. anteroposteriorly and 5 cm. transversely. Anteriorly the left half of the diaphragm is represented by a sickle shaped muscular band which disappears at about the anterior axillary line. Posteriorly a few muscle fibers arch over the esophagus as it bends to enter the stomach. This wisp of fibers then curves caudally to become parallel with those of a left crus. The crus on the right side is larger than that on the left.

At the first inspection the abdominal viscera seem to be arranged in a confused fashion, but if the point of view is taken that the median sagittal plane of reference for these organs in their typical position has undergone rotation through an angle of 90 degrees counterclockwise as the stomach, spleen, most of the intestine and half of the liver enter the thorax, then their arrangement is seen to be quite orderly (Fig. 1). Thus the falciform ligament has an almost horizontal position and the left lobe of the liver lies within the thorax. In its free (left) edge runs the ligamentum teres which enters a deep groove in the right lobe before it attaches to the wall of the abdomen. Long tongues of hepatic tissue surround the ligament in its course toward the porta. The gall bladder has its usual position on the posterior surface of the right lobe and its fossa defines one border of the prominent quadrate lobe. It is continuous with a cystic duct which joins with the common hepatic duct and comes to lie in the free edge of a lesser omentum that has its typical attachments and relations. A foramen of Winslow is present and the caudate lobe forms the roof of the lesser omental bursa. Toward the left the lesser omentum attaches to the esophagus as this structure emerges from the posterior mediastinum. The stomach is so twisted that the greater curvature is directed to the left whence arises the greater omentum. The posterior face of this diaphanous membrane has become adherent to the long transverse mesocolon, receives in its second portion the common bile duct and pancreatic duct, and is so curved that a concavity is directed toward the left, embracing the head of the pancreas. A view of this organ is obtained by incising the greater omentum to expose the floor of the omental bursa and

by dissecting at the root of the mesentery. The head of the pancreas lies at about the level of the first lumbar vertebra, much further to the left than usual. Its neck, body and tail are buried in the fatty tissues at the root of the mesentery. The duodenum becomes the first part of the jejunum by emerging from its buried position beneath the attachment of the transverse mesocolon which must be reflected upward to expose the transition. A Treitzian ligament is attached to the jejunal flexure. The small intestine lies entirely within the thorax and is of about the usual length for the newborn. It has a long fan shaped mesentery at whose root the superior mesenteric artery enters from beneath the inferior edge of the pancreas. The junction with the cecum occurs just above the costal arch. A long thin appendix arises from the cecum. Superior and inferior ileocolic ligaments and a mesoappendix are well defined. The "ascending colon" pursues an oblique course caudally and to the right but bends sharply upon itself at the hepatic flexure where the transverse mesocolon is developed. Here the relations of the large intestine and its membranes to the greater omentum have already been described. The transverse colon courses cephalically and to the left but at the splenic flexure changes suddenly to the opposite direction as it loses its mesentery. The descending colon is the most lateral structure. It is now plain that the small intestine is situated in the frame created by the large, as it were (Fig. 2). Just beyond a short free cecal pouch the ascending colon becomes attached to the sigmoid and then to the posterior wall of the abdomen by means of a membrane, as if it were in the retroperitoneum; in the thorax it is attached similarly for a short distance to the root of the mesentery, but then acquires a mesosigmoid that inserts above the root just below the twelfth rib. Laterally, above the upper pole of the left kidney, is formed the phrenicocolic shelf, and the intestine again becomes retroperitoneal within the abdomen. The sigmoid acquires a mesentery in the abdomen near the pelvis of the left kidney. Both kidneys have their usual relations to the vertebral column and ribs and lie in the retroperitoneum surmounted by the adrenals. No anomalies are noted in the ureters or in the pelvic organs. The central nervous system and cervical organs are not examined.

It is of great interest that none of the ectopic viscera have

become attached to the pleural surfaces. It is thus possible to insert the hand down the posterior wall of the thorax without meeting obstruction until the structures below the twelfth rib are reached. All of the membranes of the gastro-intestinal tract attach below this level. Molding of the liver renders restitution of the abdominal viscera difficult in gross. Were there less gas and meconium in the gastro-intestinal tract it might be possible to restore the viscera to the abdomen, despite the atypical attachments of the pancreas and root of the mesentery.

Case 2. True Hernia *

Clinical History: This white female infant was the sixth child. Four siblings are alive and well; the fifth died following an operation of unknown type performed on the 2nd day of life. The mother was 40 years of age. Her Kahn reaction was negative. The pregnancy was uncomplicated but labor was precipitate and unattended by a physician. The infant, who weighed 3625 gm., was found shortly after birth face down in a pool of bloody amniotic fluid. She was admitted to the hospital from the Outside Obstetrical Service because of cyanosis and difficulty in breathing. Both the cyanosis and dyspnea persisted during the 4 hours of life. Respirations were slow and irregular. There was a large amount of bloody mucus in the mouth and pharynx, which was suctioned out with no relief of symptoms. The chest was everywhere dull to percussion except for resonance at the right base posteriorly. Coarse loud râles were heard over the whole right side. The apex impulse of the heart was in the fourth interspace to the right of the sternum. The abdomen was doughy to palpation and the liver edge was made out to be two fingerbreadths below the costal margin. The clinical impression following the initial examination was massive atelectasis due to the aspiration of amniotic fluid. Roentgen study shortly afterward showed a diaphragmatic hernia on the left with displacement of the mediastinum to the right. Because of increasing tympany in the left axilla a stomach tube was passed which allowed air to escape under pressure, but no improvement in the infant's condition was effected by this procedure. The respirations ceased a few minutes later. The infant's temperature on admission was 94.7 F. and in spite of all efforts to raise it the temperature remained at this level during the 2 hours she was in the hospital.

Clinical Diagnoses: Diaphragmatic hernia and congenital atelectasis.

Anatomical Diagnoses: Congenital diaphragmatic hernia (left); intrathoracic ectopia of the spleen, stomach and intestine; dextrodisplacement of the mediastinum; and pulmonary atelectasis involving particularly the left lung.

Gross Examination

At autopsy the body is that of a well developed female infant, 4165 gm. in weight and 53 cm. in length. With a few exceptions

* New Haven Hospital, autopsy No. 3765.

the findings are almost identical with those of the preceding case. The ectopic viscera and the opening in the diaphragm which allows their escape from the abdomen are the same in both cases. The liver projects into the pleural cavity less than in the previous case (compare Figs. 1 and 4). Reflected over these viscera as a hood, however, and attached on the right to the stomach is a delicate membrane (Fig. 4), which continues posteriorly to invest the right face of the esophagus and a small mass of muscle which arches over this structure to form a left crus. Anteriorly it attaches on a projecting margin of the left lobe of the liver and mesially on the upper aspect of a sickle shaped lamina of diaphragmatic muscle. On the left and posteriorly the membrane inserts at about the region of the costal arch, from which it spreads as a sac toward the right preceding the ectopic viscera. It is not adherent to the pleura or to any of the abdominal organs except the stomach. The orifice of the hernial sac measures roughly 4 cm. transversely and 3 cm. anteroposteriorly. These relations make the origin of the hernial sac clear. It is probably a portion of pleuroperitoneal membrane within which muscle fibers have failed to develop (Type *2a* of Gruber). In confirmation of this view is the fact that laterally it has the attachments of the diaphragm and that a few muscle fibers are here circumferentially disposed.

Again the left lung is completely atelectatic, the mediastinum is displaced to the right of the midline, and the right lung is only partially expanded. There are no cardiac anomalies. Also, complete rotation of the intestine has occurred. The positions and relations of the stomach and spleen, and of the large and small intestine, are almost identical with those of the preceding case and the greater and lesser omentum and the mesocolon have their typical attachments.

The relations of the ascending colon are different from those in Case 1. It has no mesentery and is buried beneath the serosa of the posterior wall of the body to the right of the narrow insertion of the mesentery of the small intestine and is not attached to the sigmoid as in Case 1. Again there is a phrenicocolic ligament where the descending colon begins just above the left adrenal. The mesentery of the small intestine fans out from a very narrow line of attachment which is to the pancreas and posterior wall of

the hernial sac in the thorax, far from its usual site obliquely across the midabdomen. There is no foramen of Winslow although a definite lesser omental bursa exists. The cystic duct is thus buried in the retroperitoneum, but, characteristically, joins the common hepatic duct and enters the duodenum upon a prominent papilla immediately after union with the duct of Wirsung. A small duct of Santorini opens at a separate papilla proximal to that of the choledochal duct. The pancreas is large and is situated in the concavity of the duodenum. Its body and tail are buried in the posterior wall of the hood-like hernial sac between the stomach and descending colon rather than in the posterior wall of the body. It lies at about the level of the first lumbar vertebra. A small corner of the left lobe of the liver projects into the left pleural cavity. On one side it attaches to the sickle shaped muscular band previously described; on the other its serous surface is continuous with that of the stomach and the hernial sac, which makes a complete hood except for the foramens through which tags of greater omentum protrude. Except for distortion of the lobes of the liver by the oblique course of the round ligament the lobes and ligaments do not differ from the usual. The organ measures 11 by 7 by 4 cm. The kidneys, adrenals and the female pelvic viscera again are abdominal in position and present no abnormalities. The upper pole of the left adrenal is just below the twelfth rib.

Inspection of the fresh specimen indicates that the only difficulty in the way of reduction of the hernia is in the gaseous distention of the stomach and the meconial distention of the intestines. The small size of the herniated portion of liver makes the possibility of reduction seem more favorable than in Case 1. Despite this, ready means of closing the hernial orifice are lacking.

Case 3. False Hernia, Anomaly of Heart *

Clinical History: This white female child was born of an apparently healthy mother at what was calculated to be the 8th month. Pregnancy and labor were uneventful, the latter lasting about 12 hours. The mother's Kahn reaction was negative. At birth the child was cyanotic and did not breathe spontaneously. Artificial respiration was conducted for 51 minutes, when the heart stopped. The child weighed 1125 gm. and was 35 cm. long. External examination revealed that it was obviously premature. The palate was found to be completely cleft and hammer toes were noted bilaterally. The heart was displaced to the right but was regular in rhythm and forceful in beat. Only a

* Meriden Hospital, autopsy No. M20.

small zone above the heart on the right side transmitted breath sounds. The left side was tympanitic to percussion. On the right side of the abdomen was palpated a large mass, thought to be the liver. The child died about 1 hour after birth.

Clinical Diagnosis: Congenital diaphragmatic hernia.

Anatomical Diagnoses: Multiple congenital defects. (1) Agenesis of diaphragm (left) with intrathoracic ectopia of small and large intestines, stomach and spleen; atelectasis of left lung; and dextrodisplacement of mediastinum. (2) Persistent left and absent right superior vena cava; dextroposition of aorta; patent interventricular septum and ductus arteriosus. (3) Horseshoe kidney; cleft palate; and hammer toes.

Gross Examination

At autopsy the body is that of an obviously premature female infant weighing 1125 gm. The auricles are rounded and display an incomplete development of conchal folds. A large central defect in the palate connects the nasal and buccal cavities. Lateral ridges above the gums represent the remnants of the palate. Hammer deformities are present in the toes of both feet.

On palpation of the abdomen a firm mass filling most of the right side is felt. In contrast is the deep fossa that meets the fingers on the left. The hand can invert the skin and be introduced high into the hypochondrium on this side.

When the peritoneal cavity is opened the reason for the findings on external palpation is clear. The liver occupies most of the peritoneal cavity. Most of the abdominal viscera except the descending colon and about one-third of the loops of small intestine lie within the left thoracic cavity. They are not preceded by a hood of peritoneum but come directly into contact with the pleura on all sides. The heart is markedly displaced toward the right and the left lung is a bilobed lappet of solid dark red tissue compressed against the mediastinum. The right lung is larger but likewise is not crepitant. Patent bronchi lead from the trachea into each lobe.

The heart lies within a thin walled pericardial sac that is surmounted by a small mass of gray thymic tissue. The great vessels originate exceedingly close together at the top of the aortic arch. Remarkable changes have occurred in the heart. At the inferior angle of the huge right atrium enters the inferior vena cava. To the left of its point of entrance, guarded by a fold of endocardium, is the orifice of what is thought to be the coronary sinus. Postero-

superiorly where one would expect to find the opening of the superior vena cava, one sees only a large, thin walled blind pocket. Further inspection, however, reveals coursing obliquely upward and to the left a large tubular projection of the right atrium which becomes continuous with a left superior vena cava. This lies posteriorly of the left atrium, separated from it merely by a thin wall. The aorta is dextroposed and has an origin from the right ventricle as well as from the left. There is no foramen ovale to connect the auricles but the ductus bottali is open and a probe can also be passed from the right to the left ventricle between closely apposed bundles of muscle.

The opening in the diaphragm is oval and measures 4 cm. anteroposteriorly and 3 cm. transversely. Beginning as a shelf 2 cm. broad at the mediastinum anteriorly, muscular tissue gradually narrows as the anterior axillary line is approached. Posteriorly a much narrower ribbon of muscle crosses the esophagus and is lost behind the pleura of the posterior wall. It is therefore clear that no trace of diaphragm exists for about a third of the lateral portion. Despite the presence of most of the abdominal viscera in the thorax there are no adhesions to this membrane and none of the abdominal viscera can be considered retropleural. Pivoting about the esophagus, the stomach has turned upside down, so that its anterior (embryonically left face) is now right. A gastrocolic and gastrosplenic ligament is present. These become continuous with the serous lining of the posterior wall of the body just below the twelfth rib. The spleen thus lies cephalically of the fundus of the stomach although it has its usual attachments. There is a lesser omentum, the edge of which is free (anteriorly of a high foramen of Winslow) and contains the usual structures. A lesser omental bursa is thus formed, the floor of which is the transverse mesocolon. The first part of the duodenum runs posteriorly, inferiorly and to the right. In the crook of the duodenum which soon becomes retroperitoneal in the upper portion of the abdomen lies the pancreas. Superiorly to the duodenum through the lower edge of the pancreas runs the superior mesenteric artery. Higher courses the celiac axis whose hepatic branch enters the lesser omentum. The orifices of these vessels are about at their usual levels in the aorta relative to the vertebral column. About the superior mesenteric artery, which is longer than usual, com-

plete rotation of the intestine has occurred. The mesentery attaches along a very narrow zone to the pancreas and not at all in its usual way along a line that leads down and to the right toward the iliac fossa. The ileocecal valve lies just above the costal margin. A large vermiform appendix curves away from the tapering cecum. Superior and inferior ileocecal folds and a mesenteriolum appendicis are defined. A redundant transverse mesocolon forms the floor of the lesser omental sac as described. The descending colon becomes retroperitoneal over the left adrenal and then pursues its usual course toward the pelvis where it becomes continuous with the sigmoid. All of the pelvic viscera are of the usual size and shape and have typical relations.

The left adrenal reaches to the level of the neck of the tenth rib and lies directly superior to the kidney rather than to the right of the upper pole as usual. A serous membrane invests the anterior surface of the organ, continuous with the mesocolon which reflects upon the body wall here and with the pleura and peritoneum of the posterior wall. On the right side the adrenal extends almost to the same level as the left and bears the same relation to the kidney but the diaphragm separates it from the pleural cavity. A bar of renal tissue 1 cm. wide joins the lower poles of the left and right kidneys, creating a "horseshoe" kidney. The connecting bar is in reality an accessory lobe of renal tissue comprising a single malpighian pyramid with its appurtenant cortex drained by a projection from the left lower calyx.

A deep slit-like channel in the hepatic substance affords entrance for the round ligament. To the left of this there is on the anterior surface a deep cleft demarcating an auricle-like portion of the left lobe which, being free of diaphragm, has no "bare area." On the most medial and superior aspect of this, reflections of peritoneum from the anterior and posterior surfaces combine to form a tiny left triangular ligament which attaches to the narrow semilunar band of muscle representing the left diaphragm. There is a typical coronary ligament on the right. No notable gross changes have occurred in the liver. The major dimensions of the organ are 7, 5 and 3 cm.

The spleen is chiefly remarkable for its position relative to the stomach, but it has the usual attachment in the greater omentum which encloses it. Its diameters are increased to 5, 2.5 and 1 cm.

No difficulty is encountered in completely restoring the ectopic viscera to the abdominal cavity. Again ready means for closing the defect in the diaphragm are lacking.

Case 4. False Hernia, High Position of Left Kidney *

Clinical History: The infant was the second child of a 21 year old mother. The first child was born about 2¼ years previously and apparently developed normally. There was nothing of note in the history of the last pregnancy. The placenta was intact. Resuscitation of the newborn had to be performed. The child died about 6 hours after birth.

Anatomical Diagnoses: Congenital defect in the diaphragm (left); thoracic ectopia of the left kidney, stomach, spleen and portions of the large and small intestines; dextrodisplacement of the mediastinum; pulmonary atelectasis (bilateral); and cryptorchidism (right).

Gross Examination

At autopsy the body is that of a well developed male infant 48 cm. long. Externally there is nothing of note except for the absence of the right testis from the scrotum.

An incision reveals the mediastinum to be displaced far to the right by most of the abdominal viscera which have entered the left hemithorax through an opening in the posterior portion of the diaphragm. The left lung is a small bilobed mass of dense, airless, dark blue tissue compressed against the mediastinum, but the right lung, although likewise almost non-crepitant, contains several small bullae. What may be considered a mesoesophagus connects this thin patent tube to the aorta. The esophagus passes into the abdomen through a hiatus in the crus but then recurves over the mesial margin of the defect in the diaphragm to reenter the thorax. No cardiac or vascular anomalies are discovered and there are no unusual communications between the respiratory and digestive systems. Both phrenic nerves are present.

The diaphragm is represented by a complete dome on the right but on the left only the anterior and medial portions remain. Here the concavity of its crescent faces posteriorly. It is everywhere covered by serosa. In the region of the left anterior axillary line its muscle fibers disappear and there is no elevation of the serous lining membrane. Medially the diaphragm acts as a narrow shelf to support the left half of the pericardial sac, and posteriorly it

* Grace Hospital, New Haven Hospital autopsy No. 4747. This case was referred by Dr. C. J. Bartlett.

arches as a tiny crus over the left face of the esophagus to insert anteriorly of the left renal vein. The crus on the right side is well developed. Anteroposteriorly this defect in the diaphragm measures 2.5 cm. and transversely 3 cm.

One of the most remarkable anatomical features is the high position of the left kidney and adrenal. The adrenal extends in the midline of the back to the upper margin of the eighth rib, and the kidney to the eighth interspace. These structures can be moved only very slightly in their retroperitoneal attachments. They serve as the attachments for many of the serous membranes of the abdominal viscera (Fig. 5).

As the esophagus enters the abdominal cavity it bends cephalically and to the left and expands into the stomach. The greater curvature is now seen to be directed upward. A greater omentum attaching the spleen in a typical manner arises from the middle of the greater curvature and attaches to the anterior surface of the kidney and posterior wall of the body (Fig. 5). The attachment at first courses upward and to the left, but then swings caudally and then downward and to the right to terminate at the second portion of the duodenum. In this manner the lesser peritoneal cavity is isolated and communicates with the greater only at the foramen of Winslow, which in this case is farther to the left than usual. The lesser omentum lies approximately in its usual plane, contains the bile ducts, hepatic artery and portal vein and attaches in a typical manner to the liver. Within the crook of the duodenum lies most of the pancreas, but a small portion extends to the right of the second portion retroperitoneally (Fig. 5). When the small intestine emerges from its retroperitoneal duodenal segment its mesentery also attaches to the anterior surface of the kidney near the lower pole and proceeds cephalically and to the left. The cecum has a mesentery in common with that of the small intestine. It inserts at first along a bare area in common with that of the greater omentum. The ascending colon then proceeds in a cephalic direction and becomes retroperitoneal for a distance no greater than 1.5 cm. Then begins what corresponds to the transverse colon which is joined to the stomach by the portion of greater omentum representing the gastrocolic ligament. It is attached by the transverse mesocolon which inserts by the side of the greater omentum. The linear attachment of the mesentery of the large

intestine then curves cephalically and to the left and then forms a flexure in a caudal direction. As the region of the upper pole of the kidney is approached the large intestine becomes retroperitoneal for a distance of 4 cm., but within the abdomen again a long mesentery is acquired. The intestine does not become retroperitoneal again until deep in the pelvis where the rectum pursues its usual course.

The liver has the greatest dimension of 9 by 6.5 by 4.5 cm. On the right side it attaches to the dome of the diaphragm by a small triangular and large coronary ligament. On the left the triangular ligament is long and binds the liver to the diaphragm along a line approximately 3 mm. anterior to the crescentic posterior margin of that structure. The attachment of the lesser omentum is typical.

The right testis has not descended and is joined by a mesorchis to the posterior wall of the abdomen and is connected by means of a spermatic cord with the structures at the base of the bladder. Another anomaly is that of hypospadias since the orifice of the urethra is on the ventral aspect of the penis, 8 mm. from the distal end.

In this case there is an atypical attachment and only partial rotation of the mesentery since the first loop of jejunum is caudal in position. Since the kidney has a high position and serves as the attachment for most of the mesenteries, it is not possible to restore the abdominal organs completely below the level of the attachment of the diaphragm anteriorly although most of the left hemithorax can be cleared.

Case 5. False Hernia, High Position of Left Kidney and Adrenal *

Clinical History: This white male infant was delivered spontaneously at term but lived for only 2 hours. The family history, course of pregnancy and labor, and past history of the mother are not available. The infant was brought to the New Haven Hospital for autopsy.

Anatomical Diagnoses: Congenital defect in the diaphragm (left); intrathoracic ectopia of the spleen, stomach, pancreas, intestines, and left adrenal and kidney; dextrodisplacement of the mediastinum; pulmonary atelectasis involving particularly the left lung; and cryptorchidism (bilateral).

Gross Examination

Externally the body is well developed and 49 cm. long. When the viscera are examined the greater part of the stomach, small

* New Haven Hospital, autopsy No. 3345.

and large intestines are found in the left thorax. The diaphragm, although attached to the lateral wall of the body, has not developed posteriorly on the left side, allowing the ectopia of the viscera. The mediastinum, which contains a large thymus in its superior portion, is markedly shifted toward the right. Two small unexpanded buds represent the left lung, but aeration has partially progressed in the right lung.

The stomach is so situated that its fundus is thrown to the right, as though it had become rotated 180 degrees with the cardia as a pivot; consequently the pyloric end faces toward the left. Above the diaphragm and just to the right of the middle lies the spleen, which has its usual attachments. The liver occupies the greater part of the abdominal cavity. The left adrenal and kidney project high above the expected line of insertion of the diaphragm. The lower pole of the left kidney lies at the level of the upper pole of the right which has its usual position relative to the vertebral column. Neither testis has descended.

Case 6. False Hernia, High Position of Left Kidney and Adrenal *

Clinical History: Baby R., a male negro infant, was delivered at term spontaneously. The mother was a 30 year old negress whose Wassermann reaction was negative. There had been 9 previous pregnancies, 4 of them ending in abortion. One of the remaining 5 infants born alive died following an operation of unknown character.

The infant was 48 cm. long and weighed 2595 gm. The greatest circumference of the head was 32 cm. The infant cried spontaneously and feebly following delivery and the respirations were of a shallow gasping quality. The apex beat of the heart was made out to the right of the midline. The heart rate was slow. With stimulation the respirations and heart action improved. Three and a half hours later the infant's respirations were noted to be shallow and gasping, but improved with stimulation. Two more periods of respiratory failure followed at the 8th and 9th hours of life, from the last of which the infant could not be revived. The heart stopped 5 minutes after respirations ceased. The clinical impression was atelectasis, dextrocardia and a possible congenital cardiac anomaly.

Anatomical Diagnoses: Congenital defect in the diaphragm (left); intrathoracic ectopia of the spleen, stomach, intestines and left kidney and adrenal; dextrodisplacement of the mediastinum; pulmonary atelectasis involving particularly the left lung; and cryptorchidism (right).

Gross Examination

At postmortem the body appears well developed, weighs 2575 gm. and is 49 cm. long. The liver extends far to the left and seems

* New Haven Hospital, autopsy No. 523.

at first glance to fill the entire abdomen. A defect is present in the left dome of the diaphragm posteriorly, just to the left of the vertebral column. As in Cases 1, 2 and 3, the fundus of the stomach is directed cephalically and the esophageal and pyloric ends are in apposition. The general relations of the ectopic viscera are indicated in Figures 7 and 8. Noteworthy is the upward displacement of the left adrenal and kidney. One-third of the latter lies above the diaphragm behind the posterior wall of the body. The left lung is represented merely as a small spongy mass, but the right is partially expanded. In the left pelvic region is found a small undescended testicle.

Case 7. False Hernia *

Clinical History: Baby C., a male negro infant weighing 3400 gm., was delivered at term. The mother was a 23 year old negress whose Kahn reaction was negative. There had been 1 previous pregnancy with a living infant.

The delivery of Baby C. was precipitate. Following delivery it was noted that the breath sounds were distant and that some râles were present in both lung fields. The apex beat of the heart was noted to be to the right of the midline. After taking water well during the first 24 hours, it went to the breast at 26 hours. Immediately following this initial breast feeding it cried incessantly. The crying ceased suddenly and the baby stopped breathing. Physical examination at this time showed a cyanotic and apneic infant with a heart rate of 60 beats per minute. Artificial respiration was unsuccessful. The clinical diagnosis was not stated.

Anatomical Diagnoses: Congenital defect in the diaphragm; intrathoracic ectopia of the spleen, stomach and intestines; dextrodisplacement of the mediastinum; pulmonary atelectasis involving particularly the left lung; and cyst of upper jaw (bilateral).

Gross Examination

There is nothing remarkable in the external appearance of the child. A small cyst is found on each side of the upper jaw. Both testes are in the scrotum. Most of the peritoneal cavity is occupied by the liver and distal third of the large intestine. The other viscera have escaped into the pleural cavity through an oval opening 1 cm. in diameter in the portion of the diaphragm whose crura are intact. They displace the mediastinum to the right and so rotate the heart that the apex points anteriorly; also the left lung is compressed to a size half of that of the left. Both lungs are crepitant. The omentum is a thin membrane which covers the intestines despite their thoracic location. Just above the defect in

* New Haven Hospital, autopsy No. 2131.

the diaphragm in relation to the duodenum lies the pancreas. The spleen is in the midportion of the thorax, one side resting against the vertebral column. None of the viscera present gross anomalies.

Case 8. Herniation of Stomach Through Esophageal Hiatus *

Clinical History: Baby M., a white female, was delivered in another hospital 2 weeks after the expected date. The mother felt less well and noted that the fetus was less active than in her previous pregnancies, 2 of which had resulted in living and well infants and 2 of which ended in miscarriages. The mother's age was not stated. The infant breathed well and had a good color a few minutes after delivery. It weighed 3100 gm. It was noted after birth that the arms were externally rotated, flexed at the elbows, wrists and carpal-metacarpal joints. Full extension of the arms could be made only after persistent effort. The feet could be extended in a plantar direction to no further than a right angle with the legs. The fontanelle was almost closed and the largest circumference of the head was 32.5 cm. The length of the infant was 49 cm. The chest was prominent anteriorly.

During the first few days of life the baby occasionally became cyanotic and coughed with its feedings. Breathing at other times was irregular and occasionally there were spells of apnea. Small amounts of blood and food were vomited. Sucking was feeble. During the 1st month the cyanotic spells lessened but the cough with feedings continued. A left facial weakness, nystagmus and intermittent internal squint appeared during the 1st month.

At the age of 11 weeks it was noted that with coughing a curious gurgling sound could be heard in the region of the stomach. The history suggested a diaphragmatic hernia, but the physical signs could not be made out clinically. Just before admission to this hospital the child vomited a few cc. of brown blood and passed a large tarry stool. Roentgen study after admission disclosed a diaphragmatic hernia on the left side. During the next month she grew steadily weaker. Hemorrhages appeared in the subcutaneous tissue of the legs, hands, feet and abdomen. The night before death a large amount of blood was vomited. The clinical impression was diaphragmatic hernia (X-ray) and congenital cerebral defect.

Anatomical Diagnoses: (*Primary*): Hyperplasia of brain, involving myelin sheaths and cortex; emaciation. (*Subsidiary*): Diaphragmatic hernia; gastric ulcer; and suppurative dermatitis.

Gross Examination

At autopsy the child is 52 cm. long and weighs 2200 gm. It seems less than the stated age of 4 months. The thoracic viscera do not appear altered. The heart weighs 10 gm. and is of the usual structure.

An opening in the diaphragm at the esophageal foramen 2 cm. across permits the protrusion of the upper portion of the stomach into the thorax. The cardia is 4 cm. above the level of the dia-

* New Haven Hospital, autopsy No. 1838.

phragm. Its mucosa in large part has sloughed and the tissue which remains is dark green and obviously necrotic.

Remarkable changes are observed in the central nervous system. There is a relatively sparse amount of gray matter. Lateral and third ventricles appear dilated but the aqueduct is free of obstruction. Histologically, however, the cortex shows a normal mature cytoarchitecture. The internal capsule is surprisingly narrow and the pyramidal tracts are small. No traces of the frontopontine or of the medial portions of the corticospinal tract are discovered. In the pons transverse portions of the corticopontine and corticospinal tracts appear to be reduced in size. The entire ventral half of the spinal cord is smaller than the dorsal half.

Case 9. Eventration of Diaphragm *

Clinical History: Baby G., a white male, was delivered precipitously at term after an uneventful pregnancy. The mother had had 12 previous pregnancies, 11 of which were known to have resulted in living children, 1 of whom had a deformity of the arms and the legs. Her Wassermann reaction was negative.

The infant was admitted to the Pediatric Service when 2 hours old because of multiple congenital anomalies consisting of microphthalmus, polydactylism, syndactylism, talipes equinovalgus and cryptorchidism. On admission respirations were normal but there was some cyanosis of the extremities. Impaired resonance was found over the right side of the chest and many coarse râles were heard throughout both lung fields. The apex impulse of the heart was observed to be on the right side of the sternum. The edge of the liver was palpable one fingerbreadth below the costal margin. Roentgen study the following day showed a fractured left clavicle, atelectasis at the right apex and at the left base, the stomach lying apparently above the diaphragm on the left, and the heart transposed to the right. The infant survived for 36 days and its course was marked by a refusal to eat well, progressive loss of weight, occasional vomiting and elevation of temperature, and occasional cyanotic spells, during one of which it died. A loss of weight from 3100 gm. to 2500 gm. occurred.

Clinical Diagnoses: Microphthalmus; tongue tie; supernumerary digits (bilateral); undescended testicles; phimosis; talipes calcaneovalgocavus; dextrocardia; and eventration of diaphragm.

Anatomical Diagnoses: Eventration of diaphragm; patent ductus arteriosus and foramen ovale; interventricular septal defect; hypertrophy and dilatation of right ventricle; double ureter (right); cryptorchidism; microphthalmus; and polydactylism.

Gross Examination

At autopsy the body is found to weigh only 2500 gm. The anomalies of the extremities described in the first physical exam-

* New Haven Hospital, autopsy No. 1359.

ination are again observed. When the anterior wall of the thorax is elevated the heart is found displaced entirely to the right of the midline. The posterior half of the left diaphragm protrudes into the thorax for 6 cm. above the usual level of the dome. There is no description of the structure of the diaphragm. The stomach and the spleen which are included in the pocket appear to be normal in structure. The right ventricle of the heart is hypertrophied and composes the bulk of the organ. There is an interventricular septal defect 7 mm. in diameter and the foramen ovale is patent.

Anatomical Considerations

Embryology and Pathogenesis

Six of the 9 cases in this series are massive false hernias, 1 is a massive true hernia, 1 is an eventration of the diaphragm and 1 a hernia through the esophageal hiatus. An understanding of the location of the commonly encountered defects and of the necessary conditions in the causation and the time of occurrence of the hernias can best be gained from a review of the embryology. This has been studied chiefly by Uskow, Bochdalek, Mall, Prentiss, Keith, Lewis, Corning and Broman.

The first essential condition is the presence of a hernial orifice. The original single body cavity begins to be subdivided when the embryo is about 2 mm. long. As the heart bends ventrad, the coelom is pushed before it so that a transverse shelf, the septum transversum, is formed from the ventral wall of the body separating the developing heart from the primitive peritoneum. The ventral mesocardium disappears but the liver expands within the ventral mesogastrium as it impinges upon the septum transversum. As the cardinal veins sweep from the heart to the lateral wall of the body they produce projections into the coelom covered by mesothelium. Shelf-like expansions grow out from these in a Y shaped manner. The cephalic of these represents the pleuropericardial fold and the caudal the pleuroperitoneal fold. The former grows out to isolate the heart from the pleura. The latter in its growth narrows a channel, the pleuroperitoneal canal which connects the primitive pleural region of the coelom into which the lung buds are growing with the abdominal region. Finally during the 7th to 8th weeks a membrane is completed, the pleuro-

peritoneal membrane which separates the peritoneum from the pleura on each side. Derivatives of the dorsal mesentery and from the right lateral walls of the body help to complete the Anlagen of the mature diaphragm. The location of the pleuroperitoneal canal corresponds with that of the hernial opening in 7 of the 9 cases presented here and in many described by others.

The opening is situated dorsally, in the region of the triangle of Bochdalek, but this triangle has merely gross descriptive rather than embryological significance. The identity of location and relations of the pleuroperitoneal opening with that of the hernial orifice suggests (Gruber) that persistence of this canal is responsible for "false hernias." The variation in size is accounted for by the stage at which herniation occurs. The sizes of the hernial openings are indicated in Table I.

The mobility of the gastro-intestinal tract and therefore the relations of its membranes must be understood before the second of the essential conditions surrounding the origin of the diaphragmatic hernias (that of the presence of structures capable of herniation) can be comprehended. According to Jahn the intestines begin rotation before the time when the bowel is physiologically herniated into the umbilical region (7th week). The return to the peritoneal cavity following spacial readjustments is usually from 2 to 3 weeks after the pleuroperitoneal foramen, in the usual course of events, is already closed (Hedblom, Hofer) and retroperitonealization is not complete until the 5th month.

It is therefore obvious that either the intestines return from the umbilical pocket prematurely or that the patency of the pleuroperitoneal foramen is prolonged in most cases of congenital diaphragmatic hernia.

Only a few exact studies of the relations and attachments of the membranes in these cases (such as Grosser's or Winkler's) are available. In all 4 of those personally observed (Table I) rotation of the intestines has occurred (see Fig. 2), and partial and atypical fusion with the posterior body wall of the ascending and descending colons as well as fusion of the greater omentum with the transverse mesocolon are in evidence. This indicates the probability that the intestines were already displaced at 4½ months, when the fusions usually occur. The changes from the

TABLE 1

Anatomical Features of Interest in Four Cases of Diaphragmatic Hernia Personally Observed

	Case 1	Case 2	Case 3	Case 4
Size of hernial orifice	5 cm. transversely x 4 cm. anteroposteriorly	4 x 3 cm.	3 x 2.5 cm.	3 x 2.5 cm.
Crura of diaphragm	Present bilaterally	Present bilaterally	Present bilaterally	Present bilaterally
Phrenic nerves	Pass anteriorly of root of lung on each side	As in Case 1	As in Case 1	As in Case 1
Position of kidneys and adrenals	Upper margin of left adrenal at angle between 12th rib and spinal column	As in Case 1	Upper margin of left adrenal covered by serosa and projects up to about 10th interspace	Upper margin of left adrenal at upper level of 8th rib
Attachment of mesenteries	High and to the left of usual position	As in Case 1	As in Case 1	As in Case 1
Possibility of reduction	Much of liver projects into thorax and has a peculiar shape. Reduction would be difficult also because of distention of stomach and large intestine	Stomach distended with gas and large intestine distended with meconium would render reduction very difficult despite theoretical possibility. No adhesions to pleura	Easily possible	Not possible because of high position of left kidney to which membranes are attached

usual are in each case the failure of the mesentery of the small intestine to become attached along the oblique line low in the abdomen which passes from the left superiorly to the right inferiorly across the vertebral column. Also the partial fusions of the ascending and descending colons with the body wall are more cephalic than usual and are displaced far to the left. It is clear that a complete mesenterium commune is not in evidence as in the cases of Jahn and Liepmann, among others, and that this therefore is not a necessary condition for the occurrence of diaphragmatic hernia. It is remarkable that despite these displaced attachments fusion with the pleura has not occurred at any point. Thus, an analysis of the conditions in Cases 1, 2 and 3 reveals a theoretical possibility of restoring all of the ectopic viscera to the abdominal cavity (Table I). In Case 4 the high position of the kidney to which the membranes were attached renders this impossible.

From these embryological data it is apparent that the herniation probably occurs at some time between the 8th to 18th weeks of fetal existence. It is possible that the herniation may be before the 8th week if the duration of the "physiological umbilical herniation" is reduced by some unknown mechanism. As further evidence that the herniation occurs long before birth is the underdeveloped condition of the homolateral lung, the fact that in many instances the liver has been molded, embeds the round ligament within itself in an atypical fashion, and that occasionally the kidney, a fixed retroperitoneal structure, has also suffered upward misplacement.

If it is assumed that closure of the pleuroperitoneal foramen is delayed, what factors may be responsible? There is no hint that trauma to the mother played a role in the 4 cases personally observed, and only rarely as in cases cited by von Gössnitz and by Bischoff is the suggestion seriously considered or supported by evidence in the anamnesis. Some have regarded persistent patency of the pleuroperitoneal foramen as a consequence of some other congenital abnormality. Many (Gruber, von Gössnitz, Bischoff, Liepmann, Jahn, Grosser) have remarked on the apparently large size of the liver. To this has been ascribed the defect in the diaphragm. This idea seems illogical, however, since one would expect a large left lobe to tend more quickly to appose by means

of the coronary ligament the edges of the pleuroperitoneal opening. It is true that some livers by actual measurement are larger than usual, as in Case 2 of the present series. But Cartellieri, who examined the liver volumes in the large and well preserved collection at Innsbruck, found them in many instances to be diminished. In the presence of the low right diaphragm observed in these cases it is easy to understand how the liver may appear to be larger, even on occasion to fill the abdominal cavity when the other viscera have entered the left thorax, and yet actually be smaller than usual. In examining the livers in Cases 1, 2 and 3, as well as the illustrations from the published material of Cartellieri, Liepmann and others, the peculiarity of the course of the round ligament through the liver is striking. Usually there is a deep notch between the lobes through which the ligament passes and often a process of the right lobe extends far to the left beneath the ligament. This may unite with the left lobe. The possibility is thus suggested of some change in the growth axis of the liver, perhaps of etiological significance in producing the diaphragmatic defect. More likely, however, it may represent an adjustment to altered tensions as a result of the herniations. Kleine maintains that a high situs of the left kidney may be the factor responsible for the defect in the diaphragm. He points out that in his case the kidney was the one fixed structure among the many that were ectopic and refers to the scantiness of accurate published data in these cases. Hook and Kallius published the report of a case in which large cystic kidneys were associated with the defect. In the case of Liepmann the kidney extended to the fifth rib and in Cases 4, 5 and 6 of the present series the kidney was also high on the homolateral side. In all others of those personally observed, however, and in some described by others, both kidneys were found to occupy the usual positions. For this reason no universally pathogenetic significance can be ascribed to the anomaly. The phrenic nerves in the vast majority of cases when examined were found to be normal in origin and course but the possibility of defects in vascularization (Latarjet and Jarricot) has yet to receive detailed study. Aplasia of the lung, as in the almost unique case of Schmit, may result in a high position of the diaphragm. As shown in Table III, the most commonly associated congenital abnormality was cryptorchidism, particularly where the left kid-

TABLE II

Cases of Survival with Massive Congenital Diaphragmatic Hernias

Age	Sex	Symptoms	Course	References
yrs.				
17	M	Occasional "stitch in side." Attacks of unconsciousness	Patient struck by derrick with which he was working	Bowditch, H. I. A Treatise on Diaphragmatic Hernia. Thos. Jewett & Co., Buffalo, N. Y. 1853.
25	F	Long history of recurrent abdominal pain. Acute attack suggesting appendicitis	Operation. Reduction of large hernia. Suture of diaphragm. Cure	Borden, D. L. *Ann. Surg.*, 1922, **75**, 337–343.
33	F	Had borne 7 children. Pains in left side for several years	Immediately after overindulging in food patient seized with severe abdominal pain and vomiting	Abel, K. *Berl. klin. Wchnschr.*, 1894, **31**, 84–87.
36	M	Patient suddenly seized with sharp abdominal pain		Unger, A. S., and Speiser, M. D. *Am. J. Roentgenol.*, 1926, **15**, 135–143.
43	M	Patient working. Seized with vertigo and abdominal pain		Unger, A. S., and Speiser, M. D. *Am. J. Roentgenol.*, 1926, **15**, 135–143.
48	F	No symptoms	Patient suffered fracture of humerus. Massive hernia discovered at examination. Patient recovered	Unger, A. S., and Speiser, M. D. *Am. J. Roentgenol.*, 1926, **15**, 135–143.
43	M			Monks, E. H. *Brit. M. J.*, 1914, **1**, 708–709.
47	M		Patient died of diverticulitis of large intestine. Peritonitis	Vogel, K. M. *Am. J. M. Sc.*, 1913, **145**, 206–219.
50	M	No gastro-intestinal symptoms	Died of pneumonia of right side (left lung compressed by herniated viscera)	Thoma, R. *Virchows Arch. f. path. Anat.*, 1882, **88**, 515–555.

ney or adrenal occupied a high position. It is obvious that the association of the malformations just discussed with defects in the diaphragm is vague from the etiological point of view, is certainly inconstant, and furthermore cannot account for all cases.

The best evidence indicates that persistence of patency of the opening or failure of muscularization of the diaphragm precedes the other associated displacements and malformations. It must be admitted that a full understanding of the cause of the malformation will not be possible until the mechanical, chemical and electromagnetic determinants of typical embryogenesis are more clearly comprehended. We can at present merely define within limits the probable temporal sequence of the phenomena and thus avoid errors in establishing theories of causation.

Summary of Significant Anatomical Features

In Table I are summarized certain anatomical features of interest. In all 4 cases the mediastinum had shifted markedly to the right, the left lung had not expanded, and the intact right dome had sagged so that the liver seemed to fill most of the abdomen. Nevertheless, long life is not incompatible with the presence of large congenital diaphragmatic hernias as indicated by the cases collected in Table II. Complete reduction could actually be accomplished in the cadaver in all but the 4th of the cases dissected by the writers where the membranes were attached to the anterior surface of a kidney which projected high into the left pleural space. In Cases 5 and 6 similar conditions probably obtained although only the protocol and drawings were available for study. In all of the others the mesenteries inserted below the usual line of attachment of the diaphragm, there were no adhesions to the thorax, and the only hindrance to reduction was the presence of gas in the stomach and meconium in the intestine. So small were the vestiges of diaphragm in the 4 cases that complete closure of the ring was not possible without transplantation of muscle or fascia.

Clinical Considerations

Some of the facts of clinical interest concerning the 9 cases in this study have been collected in Table III to facilitate their discussion.

TABLE III

Clinical Data of Interest

Case number	Mother's age	Number of previous pregnancies	Abortions and miscarriages	Full term infants	Mother's Kahn or Wassermann test	Character of present pregnancy	Type of delivery	Time of delivery	Sex	Time of death	Cardinal symptoms	Cardiac apex impulse on right	Clinical diagnoses	Roentgenogram	Other congenital defects
	yrs.									*hrs.*					
1	32	1	1	0	Negative	Normal	Spontaneous	Term	M	1	Dyspnea	N.S.	? tracheal atresia	None	None
2	40	5	0	5	Negative	Normal	Spontaneous, precipitate	Term	F	4	Dyspnea	Yes	Atelectasis	Hernia (P.M.)	None
3	..	..	..	..	Negative	Normal	Spontaneous	Premature	F	1	Cyanosis, dyspnea	Yes	Congenital diaphragmatic hernia	None	Horseshoe kidneys, cleft palate, congenital anomaly of heart (left superior vena cava)
4	21	1	0	1	N.S.	Normal	Spontaneous	Term	M	6	Cyanosis, dyspnea	N.S.	N.S.	None	Cryptorchidism, hypospadias
5	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	Term	M	2	N.S.	N.S.	N.S.		Cryptorchidism
6	30	9	4	5	Negative	Normal	Spontaneous	Term	M	9	Feeble cry, periodic dyspnea	Yes	Atelectasis, dextrocardia	Hernia	Cryptorchidism
7	23	1	0	1	Negative	Normal	Spontaneous, precipitate	Term	M	27	Sudden apnea	Yes	N.S.	None	None
8	N.S.	4	2	2	N.S.	Mother less well, fetus less active	Low forceps	Term	F	4 *mos.*	Occasional cyanosis, cough with feeding	No	? diaphragmatic hernia	Hernia	Hydrocephalus, abnormalities of cutaneous blood vessels
9	38	12	1	11	Negative	Normal	Spontaneous, precipitate	Term	M	36 *days*	Occasional vomiting, refusal to eat	Yes	Atelectasis	Hernia	Polydactylism, syndactylism, clubbed feet, cryptorchidism, microphthalmus, congenital anomaly of heart (patent interventricular septal defect)

N. S. = not stated.

Of the 6 mothers whose ages were stated, 4 (Cases 1, 2, 6 and 9) were in the latter half of the child-bearing period, being 30 years or more of age at the time their offspring with congenital diaphragmatic hernias were born. Seven mothers were multiparas (Cases 1, 2, 4, 6, 7, 8 and 9). It is impossible to say from this small group of 9 cases whether the occurrence of these anomalies was associated in any way with the age or parity of the mothers. In order to obtain further information concerning the incidence of congenital diaphragmatic hernias according to the age and the number of pregnancies of the mothers, 25 additional cases were picked at random from the literature. Of the 34 mothers only 12 had their ages recorded. Five of these were under, and 7 were over 30 years of age. In the general population the ratio of mothers under 30 to those over 30 years in the child-bearing period is about 10 to 3. Apparently the incidence of these anomalies is suggestively higher among mothers of 30 or more years of age than among those under 30. The ratio of 6 primiparas to 17 multiparas who bore infants with congenital diaphragmatic hernias is similar to the ratio of primiparas and multiparas in the general population. The parity of the mother is probably of no significance so far as the incidence of infants with these congenital defects is concerned.

From Table III it can be seen that 7 mothers (Cases 1, 2, 4, 6, 7, 8 and 9) had had a total of 33 previous pregnancies; 8 of these ended in abortions, giving a ratio of 1 abortion to every 3 infants born alive, which is similar to the abortion ratio in the general population.

The pregnancies and labor of the 9 mothers were not remarkable except in Case 8. The mother of this infant felt less well during her pregnancy and noted that the fetus was less active than in her previous pregnancies.

Discussion of the symptoms of these hernias and their relation to the pathology needs no enlargement after the excellent description by Greenwald and Steiner. It is to be noted that in only 1 (Case 3) of the 9 infants were the symptoms and physical findings correctly diagnosed clinically without the aid of roentgenography. In 4 other cases the *intravitam* diagnosis was made by roentgen study (Cases 2, 6, 8 and 9) and in the remaining 3 (Cases 1, 5 and 7) the anomalies were first discovered at the

postmortem examination. The incidence of *intravitam* diagnosis in this group of 9 cases is similar to the experience of others. Greenwald and Steiner in reviewing 82 cases in children under 10 years of age found that the diagnosis was made clinically in only 6, that in 21 others it was made by roentgen examination, and that in 43 the defect was first detected at the autopsy table. Truesdale's more inclusive survey of 303 cases in children disclosed that in 165 of them the diagnosis of a hernia was made only at autopsy. Some of the difficulty in diagnosing these cases clinically is probably due to the high mortality (about 50 per cent) during the first few hours of life, permitting insufficient time for clinical observation. Seven of the 9 infants in the present study died within 27 hours of birth (Cases 1, 2, 3, 4, 5, 6 and 7) and 4 of them died within 4 hours (Cases 1, 2, 3 and 5). Forty-three of the 82 cases reported by Greenwald and Steiner, or over half, were either stillborn or dead within 24 hours of birth. This lack of sufficient time for clinical observation is not, however, the whole difficulty in diagnosis. Part of the latter is due to the failure of the clinician to consider these anomalies in the differential diagnosis when confronted by an infant with respiratory difficulty and with the cardiac apex impulse on the right side of the chest. In 5 infants (Cases 2, 3, 6, 7 and 9) the apex beat of the heart was located to the right of the midline. Although the clinician does consider congenital diaphragmatic hernia in differentiating these anomalies from other cardiorespiratory and gastro-intestinal disorders, it is sometimes impossible to correlate the physical findings with the symptomatology. Physical examination may be entirely negative in the presence of extensive hernias. Such was the case in the 8th infant of this series. The history of occasional cyanosis, cough with feeding, vomiting of blood and food, and a curious gurgling noise located in the region of the stomach when the child coughed made the clinician suspect a diaphragmatic hernia, but the physical findings in the chest were entirely negative. The diagnosis was subsequently made by roentgen study.

The discussion in the literature of the clinical aspects of these anomalies has been largely an academic one. Up to 1935 only 17 infants under a year of age had been operated on, 9 of them successfully. The fact that the youngest of the 9 was less than 48 hours old at the time of operation would indicate that the age of

the patient was not a contraindication to surgical procedures being carried out. In retrospect it would appear that 2 of the infants (Cases 6 and 7) might have benefited by operation had the significance of the "dextrocardia" been appreciated earlier, for the defects in the diaphragm of both infants were only partial and the clinical course of each infant was not so stormy but that an operation might have been at least considered. Probably only by routine clinical examination immediately after birth and an increase in the incidence of correct clinical interpretation of "dextrocardia" will such infants in the future have the benefit of at least a surgical consultation. No attempt was made to correct the diaphragmatic defects in Cases 8 and 9, where the infants survived for 4 months and 36 days respectively, because of the extensive congenital defects present in each infant in addition to the diaphragmatic anomaly.

The high incidence of anomalies other than those present in the diaphragm of these infants has been noted in many previous reports in the literature. Six infants (Cases 3, 4, 5, 6, 8 and 9) had additional defects. Four had cryptorchidism which was noted at postmortem examination and four had multiple defects as noted in Table III.

Summary

A study of available data indicates that in most instances a persistence in patency of the pleuroperitoneal foramen beyond the 7th to 8th week of intrauterine life is probably responsible for the defect known as congenital diaphragmatic hernia. The one associated anatomical change found in all 4 specimens we dissected was in the atypical rotation and attachment of the mesenteries. This suggests that the intestines were already misplaced at the middle of the 4th month, the usual time of rotation and fixation. It is concluded that the high position of the left kidney observed three times in the present series cannot invariably be the cause of the defect in the diaphragm. The abdominal viscera are attached in the thorax only where the kidney in a high position serves as the attachment of the mesenteries.

Some of the clinical aspects of these cases have been considered. The difficulty in diagnosis during life by clinical observation is stressed and the reasons discussed.

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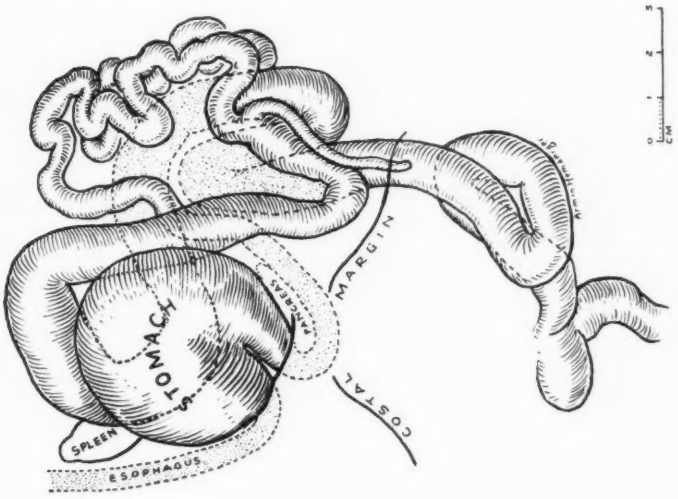
DESCRIPTION OF PLATES

PLATE 125

FIG. 1. Case 1. Congenital defect in the diaphragm (left). Thoracic ectopia of the abdominal viscera. Note the position of the liver and atypical course of the round ligament.

FIG. 2. Case 1. Complete rotation of the mesentery and intestine. An atypical high attachment of the mesentery is seen.



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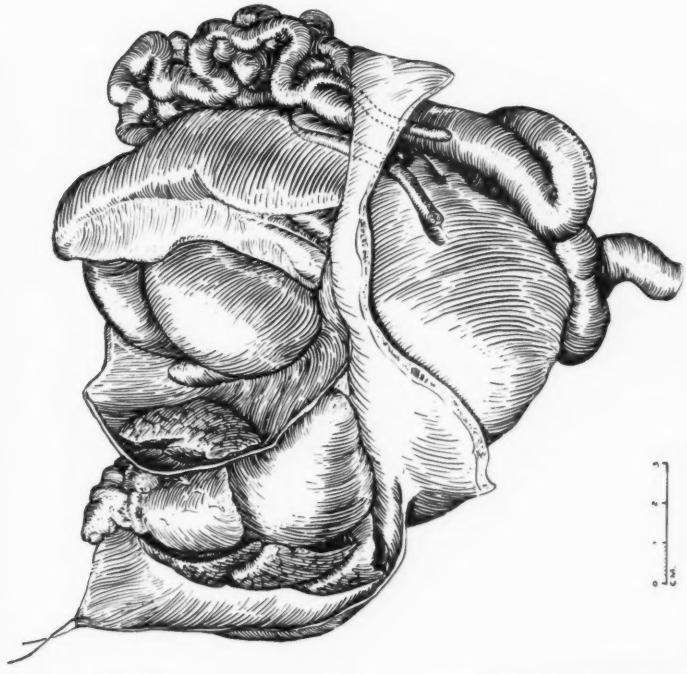

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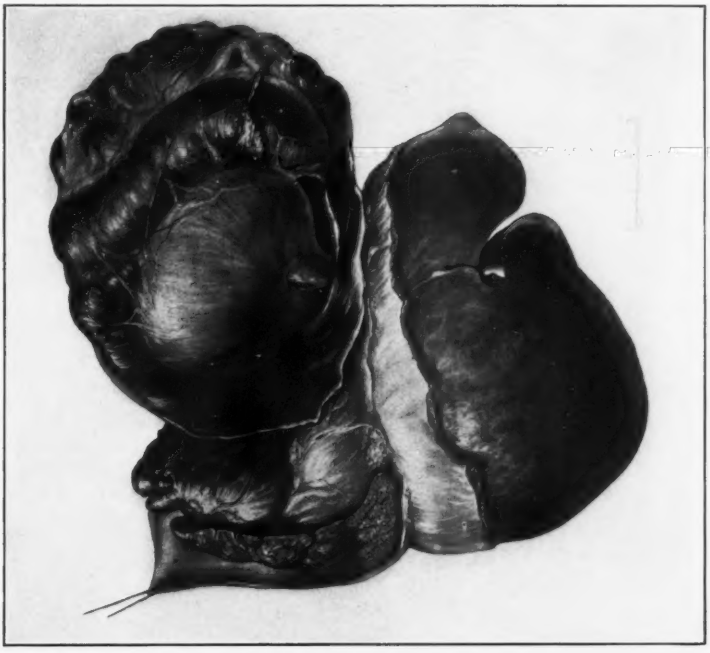
PLATE 126

FIG. 3. Case 2. Roentgenogram (postmortem). The inverted stomach is seen in the left thorax. Displacement of the viscera to the right is present. The thin barium fluid outlines also the bronchial tree.

FIG. 4. Case 2. True diaphragmatic hernia. A complete hood of membranous diaphragm precedes the ectopic abdominal viscera. Note the penetration of a portion of the greater omentum through a foramen in this membrane.



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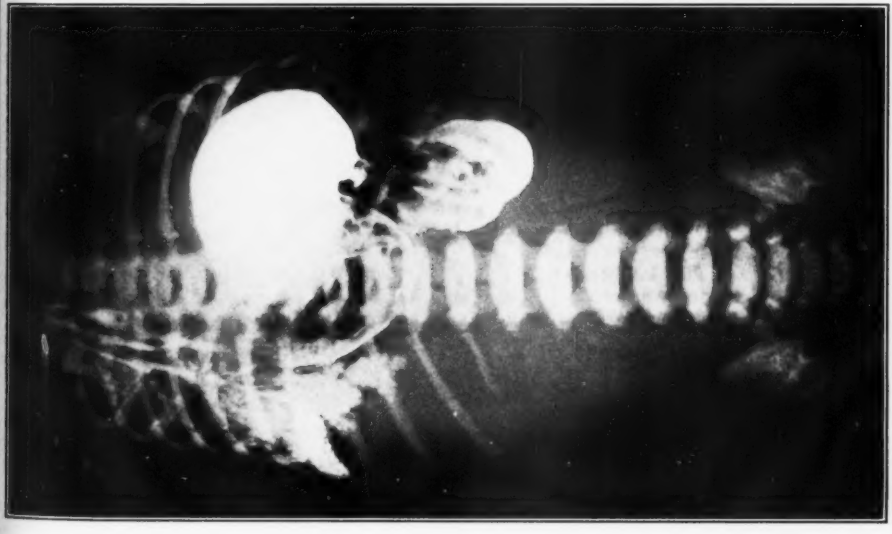


3

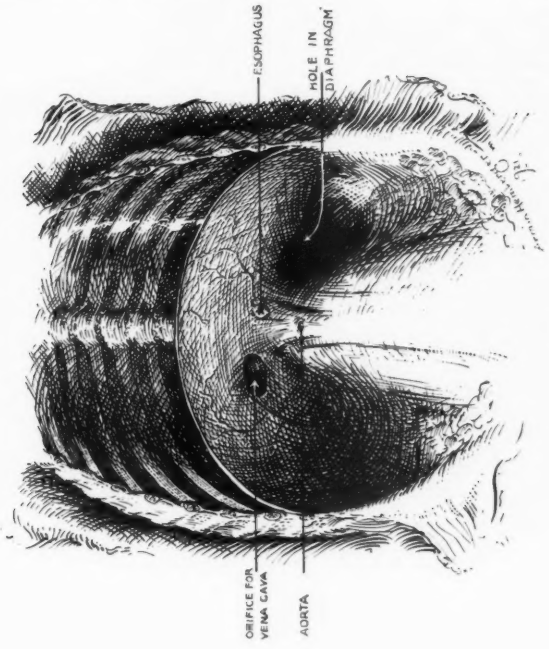
Liebow and Miller

Congenital Defects in Diaphragm

PLATE 127

FIG. 5. Case 4. High position of the left kidney. Note the attachments of the mesenteries to the anterior surface of the kidney and posterior body wall. From left to right the vertical lines of attachment represent (1) greater omentum; (2) greater omentum together with ascending and transverse mesocolon forking on the left to the mesentery of the small intestine, and on the right to the greater omentum; and (3) descending mesocolon. Note the projection of the head of the pancreas to the right of the duodenum.

FIG. 6. Case 6. Defect in the diaphragm. Note the posterior location of the defect in the region of the pleuroperitoneal foramen.



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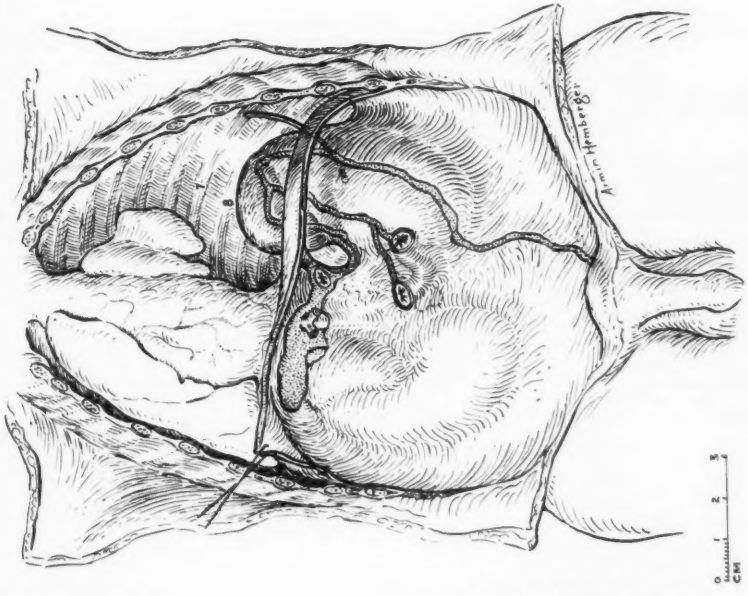


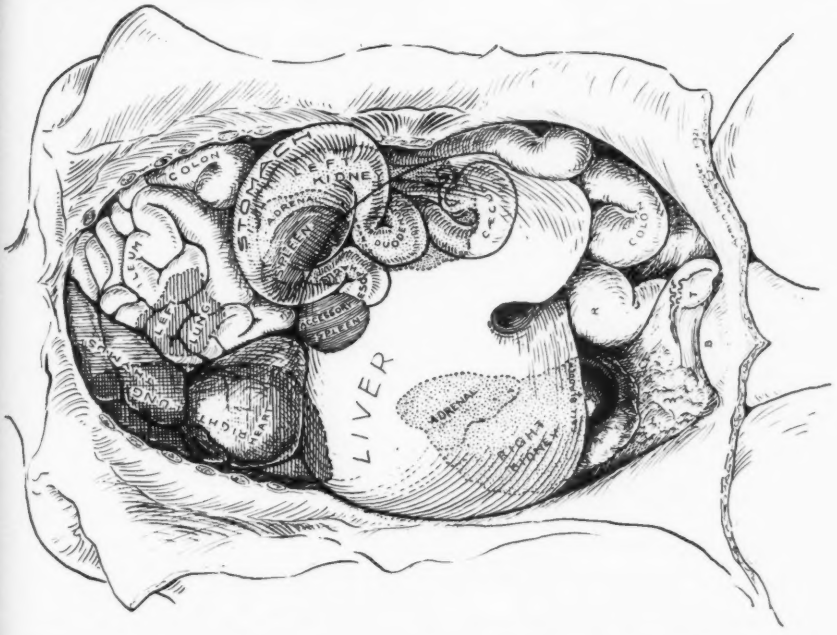
5

Liebow and Miller

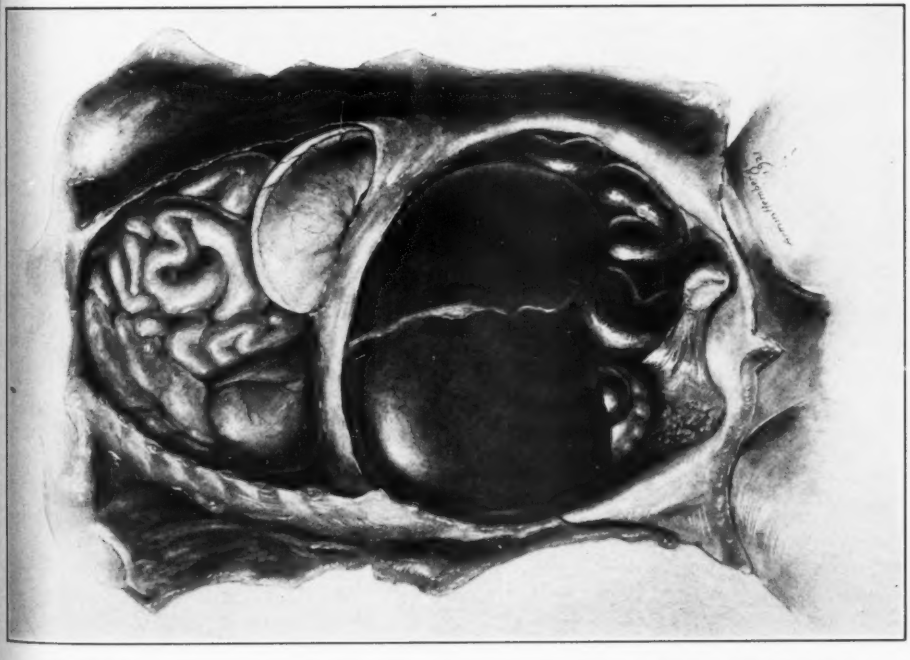
Congenital Defects in Diaphragm

PLATE 128

FIGS. 7 and 8. Case 6. Note the high position of the left kidney and the non-descent of the testis.


STOMACH
LEFT KIDNEY
COLON
ADRENAL
SPLEEN
DUODENUM
CECUM
COLON
JEJUNUM
KIDNEY
GALL BLADDER
LIVER
ADRENAL
RIGHT KIDNEY
LEFT LUNG
HEART
RIGHT LUNG

8



7

THE CYTOLOGY OF FOX ENCEPHALITIS AND THE EFFECTS OF CENTRIFUGATION UPON THE INTRANUCLEAR INCLUSIONS *

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The purpose of this investigation has been to ascertain: (1) the succession of stages produced by the virus of fox encephalitis in the nuclei of reacting cells; and (2), by the use of the centrifuge, something about the specific gravity of intranuclear inclusions relative to other constituents of the nuclei.

The epidemiological and immunological aspects of fox encephalitis, the pathology of damaged tissues and the distribution of inclusions may be found in papers by Green and his associates.[1-7]

MATERIALS AND METHODS †

The tissues selected for study were the brain and the liver. Corrosive sublimate and acetic acid, as given by Cowdry for the Feulgen technic,[8] and Zenker's fluid plus acetic acid were the fixatives used.

The Beams ultracentrifuge employed and the method used to detect possible autolytic effects during centrifugation were the same as described in preceding publications.[9, 10] In the present study, as well as in previous ones, these controls have indicated that no significant autolytic effects took place which could be attributed to the lapse of time between removal of the tissues and their fixation. Evans [11] observed in tumors kept at room temperature, in the ice box and in the incubator, that the number of mitotic figures remained the same for as long as 24 hours after removal of the tissue. Mallory,[12] from his studies on histopathol-

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† This investigation was made possible through the invitation and facilities offered me by Dr. R. G. Green of the Department of Bacteriology, University of Minnesota Medical School. He inoculated the virus of fox encephalitis into the cisterna cerebellomedullaris of young red foxes several days before my arrival, and during the three days spent in his laboratory collecting materials from sick animals I had the generous assistance of Dr. C. L. Larson.

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ogy, considers postmortem tissues fixed within 1 to 2 hours after death as fairly trustworthy.

It was observed after the work was under way that the air used to drive the centrifuge was warmer than the surrounding air. The effects of the high temperature are evident on many of the centrifuged tissues so that some of the pieces had to be discarded and those which were sufficiently normal to use in this study are interpreted with this in mind.

Most of the information was gained from the use of azure-eosin and the Feulgen reaction.

Observations

In the tissues of the central nervous system inclusions occur in ependymal cells, in cells of the lamina epithelialis, and in endothelial cells of blood vessels, but they are absent from nerve and glial cells. The tissues affected by the virus are often injured to the extent that extensive damage is frequently observed. The occurrence of necrosis in both inclusion and non-inclusion-bearing cells has been very helpful in that it is possible, by making comparisons between the two, to distinguish the particular cell changes which should be assigned to autolytic effects or to the direct action of the virus. The descriptions which follow are based largely upon cells of the lamina epithelialis of the tela choroidea because here one may study easily a sufficient number of affected cells. Very little information could be obtained concerning the process of inclusion formation in the liver because in that organ frequently much searching was necessary in order to find one inclusion-bearing cell. At the end of this section of the paper is discussed the interpretation of these data as applied to ependymal, endothelial and fibroblast cells which bear intranuclear inclusions.

The "Normal" Nucleus as Seen in Fixed Material

The cells of the lamina epithelialis possess nuclei which are relatively large, oval in shape, and in which the chromatin is present as small granules uniformly dispersed throughout the nucleoplasm (Fig. 1). A faint linin network joins the granules together. No nucleolus is present but in some cells a few chromatin granules have clumped together to form a small irregular body.

Necrosis in Non-Inclusion-Bearing Cells

The sequence of stages leading to pyknosis has been arrived at by an examination of a great many nuclei undergoing disintegration and then arranging them in what is regarded as the correct order. Many pitfalls are known to attend such a method. In the case at hand, however, we have a good idea of what to expect at the two ends of the series, namely the normal resting nucleus and the pyknotic nucleus ready for the last steps in the process of disintegration. A previous but unpublished study on the cytology of the nucleus in healthy rats and pigeons during postmortem autolysis has been helpful in arranging the sequence, and also the careful study by Schmaus [13] on karyorrhexis.

The first reaction of the nucleus is an increase in the number of chromatin granules (Fig. 2). The particles remain uniformly dispersed and are only slightly larger than those of the normal nucleus. Frequently, either before hyperchromatination begins or in the early stages of the process, there appears to be a slight increase in size of the nucleus as a whole. This may take two forms, either a uniform expansion of the nuclear wall or lobulation. Following the early reactions the granules do not remain small; instead, they fuse together to form larger spheres and, of course, a decrease in number of granules accompanies this reaction (Fig. 3). At the same time these events are taking place the nuclear sap develops a positive Feulgen reaction which would indicate that some of the chromatin * had undergone solution. As liquefaction takes place the nucleus decreases in size, the nuclear membrane shrinks, and the contents of the nucleus are crowded together, with the result that the chromatin is reduced to a few large spheres. The result is the typical, shrunken pyknotic nucleus in which the particulate and dissolved chromatin forms a mass which stains deeply and is homogeneous except for vacuoles (Fig. 4). Beyond this point the changes could not be traced with sufficient certainty to lead to any opinion about them.

* I am aware that Feulgen's reaction is a test for thymonucleic acid and not for chromatin *per se*, and that not all chromatins are Feulgen-positive, as pointed out in the earlier works of Feulgen and Rossenbeck,[14] Verne,[15] in investigations on protozoa by Lucas,[16] Lucas and Evans,[17] and in a comparative study of technics by Carlson [18] on corn. Yet in numerous publications dealing with mammalian tissues basophilic material within the nucleus has been recorded as Feulgen-positive. The tacit assumption has been made in this paper, therefore, that the Feulgen reaction is a reliable indicator of the presence of basichromatin.

Nuclear Reaction and Necrosis in Inclusion-Bearing Cells

The question has occasionally been raised, "What is a reliable criterion for determination of the relative age of an inclusion body?" Frequently the stages have been selected on the basis of size of the inclusion body, the smallest being considered the youngest. In this work and in a previous study of intranuclear inclusions of submaxillary gland disease of guinea pigs,[10] it was noted that the size of the inclusion body is an inaccurate and misleading criterion of age. The nuclear reaction proved to be a more reliable indicator of age but is somewhat more difficult to evaluate.

As the study of fox encephalitis progressed it became evident that there were distinguishable two series of changes taking place in cells which develop intranuclear inclusions: (1) the reactions which are brought about by the presence of the inclusion; and (2) the reactions which accompany the necrosis of the cell which is the effect of the action of the virus on the tissues as a whole and probably not the direct effect of the presence of the inclusion body. It was not until these two factors had become apparent that it was possible to arrange the various stages in what is now regarded as their probable sequence.

The chromatin granules are usually small in the earliest recognizable stage of inclusion formation (Figs. 5 and 13). They are uniformly scattered, but probably not as uniform in size as in the normal nucleus. Condensation of chromatin on the nuclear membrane is not apparent but there is a tendency for the linin network and its chromatin to pull away from the inclusion body, which reaction produces structurally empty spaces in the regions of the inclusion but not a well formed halo. The inclusion body from its earliest recognizable beginning is Feulgen-positive and basophilic after azure-eosin.

As margination takes place a reaction occurs which has been observed only in fox encephalitis and which is absent in submaxillary gland disease of guinea pigs. It is found in the oxychromatin component of the nucleus. This material separates from the basichromatin and then migrates toward the Feulgen-positive inclusion body. It is colored green with the light green counterstain (Figs. 6, 7 and 8) and pink in azure-eosin (Fig. 14). Its migration and subsequent adherence to the inclusion body may

be followed. The stage shown in Figures 7 and 14 is quite common, but the interpretation of the acidophilic covering over the inclusion body was not possible until the stage shown in Figure 6 was found.

About the time the oxychromatin has reached the inclusion body the basichromatin has fully marginated and in optical section forms a thin Feulgen-positive line against the nuclear membrane (Fig. 7). The view in optical section, however, is not the true picture and when one focuses vertically on the surface of the nucleus it is evident that the chromatin does not coalesce to form a shell of uniform thickness but remains as small separate granules placed close together.

In later stages the chromatin does not persist as an apparent thin line, when observed in optical section, but appears to break up into short rod-like segments of uniform length (Fig. 8). The space between the segments is less than the length of the segment. The true picture, as before, is revealed in a vertical view of the surface of the nucleus; it is then noted that the chromatin has aggregated into masses which are larger than the granules of earlier stages.

The process of coalescence continues; the masses become still larger and the spaces between them become greater (Figs. 9, 10 and 15). Those portions of the nuclear membrane left bare by the contraction of basichromatin are Feulgen-negative and do not have a strong affinity for the counterstain so that it is usually difficult to determine whether the nuclear membrane has remained unbroken, but its subsequent shrinkage would indicate that it was intact and acting as a semipermeable membrane. The nuclear membrane appears to be stained quite clearly after azure-eosin and is acidophilic. During this period of basichromatin change the oxychromatin which had previously joined the inclusion body becomes less easily distinguishable from the inclusion substance. When the oxychromatin was at first separated from the basichromatin it was Feulgen-negative and stained with the light green counterstain, but as it takes a position at the periphery of the inclusion body it is colored gray after the use of Feulgen and light green. It was concluded in this case, as in the submaxillary gland virus investigation,[10] that two substances, one staining red and one green, when mixed together produced the gray color and

that the gray color does not indicate a new material but a mixture in which the original staining affinities have been retained. The same gray color is produced in azure-eosin; in this case, also, complementary colors are combined (Fig. 15).

Frequently strands extend out from the oxychromatin to the nuclear membrane (Figs. 7–9 and 14–17). These are Feulgen-negative and acidophilic. They seem to be continuous with the oxychromatin but it is doubtful if the two substances are identical and the reasons for this opinion will be set forth when the centrifuged cells have been considered (p. 748).

Up to this time the series shows very little variation, but from this point autolysis superimposes its own course of events on the picture and the series will be modified, depending on the time when autolysis strikes. If autolysis comes at the time of complete margination of chromatin, for example, at such stages as represented by Figures 7–9, then the sequence from that point will follow the course shown in Figures 10 and 11. If the nuclear reaction is allowed to run its course then there is obtained a sequence such as that shown in Figures 15, 16 and 17.

In the former case, where autolysis comes early, the first obvious effect is the shrinkage of the nuclear membrane. It goes as far in the process as the inclusion will permit, with the result that the halo is obliterated (Figs. 11, 12 and 18). During the shrinkage process or before it begins the basichromatin masses reach their maximum size and minimum number. When an inclusion body of medium size is present the chromatin clumps are relatively close together (Figs. 11 and 18), but when the inclusion is large, as in Figure 12, the clumps are far apart, which would indicate that the amount of chromatin surrounding the inclusion remains approximately constant and is independent of the size of the inclusion body. The nucleus, therefore, exclusive of the inclusion body, is showing reactions characteristic of pyknosis. The end result is a small nucleus with the basichromatin clumps pressed against the combined oxychromatin and basophilic inclusion body. The oxychromatin does not retain its identity with increasing age; the entire inclusion body becomes Feulgen-positive. It is not known whether the oxychromatin disappears or has become so dispersed that it no longer has an influence on the red color of the Feulgen reaction or the blue of azure-eosin. The only reaction shown by

the inclusion itself to ageing and necrosis is loss of homogeneity which is apparent by the formation of vacuoles.

If the inclusion-bearing cell does not undergo necrosis then the nuclear reaction to the inclusion body can proceed to completion. This process was discovered in azure-eosin stained slides; Feulgen material was not suitable in that the nuclear wall is almost invisible when free from chromatin. Numerous examples were located in which, after the marginated basichromatin had clumped to its maximum degree, some of the masses had partly pulled away from the nuclear membrane (Fig. 15). All degrees of central migration of basichromatin were noted; some were just starting, others were midway, and many had reached the central "inclusion-oxychromatin" mass (Fig. 16). In this migration the basichromatin usually follows the acidophilic strands which extend from the oxychromatin to the nuclear membrane.

At the final stage in the reaction all of the basichromatin clumps have applied themselves closely to the surface of the inclusion body (Fig. 17). The halo at this stage lies between the basichromatin and the bare nuclear membrane. A few examples were found which indicate that the sequence of stages in necrosis from this point on probably follows the same course as already described for inclusion-bearing cells, in which steps leading to pyknosis begin at the time of margination, namely, loss of fluids and shrinkage of the nuclear membrane until the latter reaches the central mass. The end result is almost indistinguishable from that in which the shrinkage of the nuclear membrane is directly responsible for bringing the basichromatin against the inclusion body. These stages in autolysis were found affecting nuclei, such as Figure 16, in which the process of central migration had only begun, and occasionally it was observed acting on cells which had reached the stage shown in Figure 17.

Interpretation of These Data Applied to Other Inclusion-Bearing Cells

Inclusions occur in various cells which can be brought together into two main groups; one includes the cells of the lamina epithelialis and the ependyma, and the second includes the endothelial cells, monocytes and macrophages.

The ependymal cells pass through all the major changes de-

scribed for inclusion formation in the lamina epithelialis. In most of the cells the inclusion body is large and margination is complete, but occasionally early stages may be found in which the chromatin has not completely marginated and, likewise, late stages in which the chromatin has clumped. Only rarely does one observe the return migration of basichromatin to the inclusion body.

The observation by Green, Katter, Shillinger and Hanson [4] that the inclusion bodies in ependymal cells show a variation in staining from acidophilic to basophilic has been confirmed in this study and the same interpretation is applicable that was given for the cells of the lamina epithelialis, namely that the inclusion body is made up of two elements, one a basophilic Feulgen-positive material, and second an acidophilic portion which is the oxychromatin of the nucleus. The completely acidophilic inclusion is interpreted as one in which there has been only a very small basophilic body which has become invisible because it had been completely dispersed through the oxychromatin and its proportion is too small to influence the color reaction of the acidophilic dyes used. This opinion is supported by results with the Feulgen technic in that the reaction is very faint and the material present is dispersed throughout the oxychromatin. The inclusions which reveal a distinct basophilic and Feulgen-positive center and an acidophilic mantle are interpreted as two substances which have not mingled but have remained separate from each other. The thickness of the basophilic inclusion and oxychromatin layer in such examples is variable. The basophilic Feulgen-positive inclusion is interpreted as one in which this material is proportionally greater than the oxychromatin and is diffusely scattered so that the oxychromatin is masked. All of these effects lead to the conclusion that an acidophilic and a basophilic inclusion are fundamentally alike; their differences merely represent differences in proportion of two stainable materials and differences in degree of mingling of the two substances. Such wide differences in staining would indicate that tinctorial reactions in themselves are of little value but tinctorial effects serve a very useful purpose when the fundamental components which bring about the reactions have been identified and followed through the evolution of the process.

The ependymal cells present another point of interest in that

there frequently occurs a clump of chromatin granules in the resting nucleus which is about the same size as the basophilic center of some inclusion bodies, which suggests the possibility that the two might be identical. That this is not the case is shown by those cells in which both the clump of chromatin granules and the basophilic inclusion can be identified. The former marginates with the other chromatin particles. Therefore, it must be concluded from data available at the present time that the basophilic center of the inclusion body is of unknown origin and cannot be traced to any formed constituent of the nucleus.

Occasionally one ependymal cell in a row of cells will undergo necrosis and the course followed is similar to that given for cells of the lamina epithelialis, namely, liquefaction of basichromatin followed by shrinkage of the nuclear membrane and loss of nuclear fluids.

Fibroblasts rarely develop inclusions and not enough examples were observed in order to follow the course of events, but the fact that they possess a well developed plasmosome nucleolus surrounded by chromatin granules makes it worth while to record the fact that the whole nucleolus marginates with the basichromatin when an inclusion develops in the nucleus; apparently there is no tendency for the plasmosome to separate from the surrounding chromatin and move toward the inclusion body.

Superficially the inclusions in liver cells resemble those in the lamina epithelialis but the two types are fundamentally different. The inclusions in liver cells have a uniform composition although vacuoles may be present, whereas the inclusions in the ependymal cells and especially in the lamina epithelialis possess a basophilic center and a mantle of oxychromatin. In liver cells small inclusions associated with early stages in nuclear reactions in which the chromatin is beginning to marginate show no indication of oxychromatin separation from basichromatin or its migration toward the inclusion body. The inclusion body usually stains various shades of gray with azure-eosin and is slightly Feulgen-positive. The intensity of basophilic staining is greater in old stages than in young ones.

The nuclear reaction, as far as it was traced, is quite similar to that shown by the cells of the lamina epithelialis. A prominent

nucleolus is present in the liver cells and this, during the margination process, is repelled with the other chromatins.

Centrifugation Studies

Centrifugation of the normal resting nucleus of the lamina epithelialis causes the basichromatin to aggregate at the centrifugal pole (Fig. 19). The particles are pressed together in a compact mass and only rarely does one find a fleck of basichromatin left scattered in other parts of the nucleus. The centrifugal force causes the mass of basichromatin to bulge the nuclear membrane, usually only a slight amount, but sometimes it produces a characteristic dumb-bell shape, as in Figure 19. Investigators who have centrifuged egg cells and plant and animal tissues have observed many times a constriction and division of the nucleus, and in mammalian tissues, and usually in plant tissues, the nucleolus is the heaviest, the chromatin next, and the nuclear sap the lightest. However, in the germinal vesicle of the ascidian egg the nuclear sap is heaviest and the chromatin lightest.[19] In the present instance the high centrifugal force has brought about in addition a separation of two substances in the nucleus, the basichromatin and oxychromatin. The separation and stratification of the two chromatins has not previously been recorded as far as I can discover. The oxychromatin forms a homogeneous layer on the centripetal side of the basichromatin and is closely pressed upon it. It usually arranges itself to conform to the available spaces and forms a flat surface next to the nuclear sap. The oxychromatin, therefore, has a lower specific gravity than the basichromatin.

In centrifuged nuclei strands of acidophilic material extend from the nuclear membrane on the centripetal side to the oxychromatin. Such strands usually occur in plant and animal cells and have been called linin substance. The material is tenacious and obviously viscous and is strongly attached to the nuclear membrane. These properties are different from those of oxychromatin and basichromatin. Its affinity for acidophilic dyes is the same as the oxychromatin and only by its physical properties can it be distinguished from oxychromatin. It is these observations which form the basis for the statement on page 744 that in inclusion-bearing cells the acidophilic material which migrates

centrally from the marginating chromatin is oxychromatin and that the strands which extend from this material across the halo to the nuclear wall are linin substance.

Centrifugation of inclusion-bearing cells reveals several points of interest (Figs. 20 and 21). The basichromatin, as before, is the heaviest substance in the nucleus. The inclusion body comes to rest against the basichromatin. It sometimes causes the nuclear membrane to bulge. Since the inclusion is composed of two parts, the basophilic center and the mantle of oxychromatin, the question arises, "Will these two constituents show a tendency to separate when centrifuged?" Apparently they do. It is not shown in every inclusion (Fig. 21) but it would not be expected in those inclusions which in the uncentrifuged cell stain uniformly densely, for example inclusions of the type shown in Figures 9, 10 and 16; but in cells in which the oxychromatin has more recently joined the inclusion body, for example Figures 7, 8, 14 and 15, then centrifugation leads to an asymmetrical distribution of oxychromatin (Fig. 20). The Feulgen-positive material of the inclusion moves toward the centrifugal pole and the oxychromatin part toward the centripetal pole, and thus the oxychromatin takes the same orientation in relation to the basophilic inclusion that it does to the basichromatin in the normal nucleus.

Several cells were found in which the basichromatin had returned from the nuclear wall and had joined the inclusion body. Centrifugation did not separate the basichromatin from the inclusion body. When the amount of basichromatin which adheres to the inclusion is great then the nucleus contains only a small amount of free chromatin which can be concentrated at the centrifugal pole (Fig. 21). These reactions are identical with those observed when submaxillary gland duct cells of guinea pigs infected with submaxillary virus have been centrifuged.[9, 10]

The normal non-inclusion bearing cells of the liver react about in the same way as those in the brain, in that there is stratification of the chromatins and the oxychromatin lies centripetal to the heavier basichromatin. Only a few inclusion-bearing cells were located in the liver. When the inclusion was small it was usually not displaced at all from its original position at the center of the nucleus and this was true also of larger inclusions if the linin strands were abundant (Fig. 23). These strands from the

inclusion to nuclear wall did not prevent, however, the slipping of the chromatin to the centrifugal pole. When the inclusion body is displaced by centrifugal force it moves through the nuclear sap and comes to rest against the heavier basichromatin (Fig. 22). Thus it is lighter than the chromatin and heavier than the nuclear sap and in this respect it resembles the inclusions in the cells of the ependyma and lamina epithelialis.

Discussion

Cowdry, from a survey of his very extensive collection of slides, contributed the first definite classification of inclusions when he suggested Types A and B.[20] This was restated by Cowdry, Lucas and Fox [21] the following year. However, since the time of these publications intensive study on some of the inclusions makes it desirable to set forth a little more exactly the criteria used for classification and to shift some which were at first in Type A from A to B.

Type A inclusions are those composed of particulate bodies which are approximately spherical and of uniform size. The shape into which the granular bodies are clustered may take any form. Growth of the inclusion takes place by the addition of new particles to the group. In all known examples they are acidophilic in their staining reaction. These criteria apply to early and intermediate stages of inclusion formation because late stages frequently produce coalescence of the granules to form one or several, large, apparently homogeneous spheres or irregular masses, or crystal shaped bodies which superficially may resemble Type B inclusions. An example of this is shown in the kidney of the Guatemalan amazon.[21] At the present time it seems best to exclude Cowdry's generalizations made in regard to the nuclear reactions, namely the degree of margination and the tendency to partial or complete degeneration because, for most of the viruses, too little detailed information is available concerning all of the phases in the reaction series, and most accounts have not distinguished between the intrinsic reaction of the nucleus to the presence of an inclusion body and the generalized tissue reaction which the virus may produce independent of inclusion formation.

Type B inclusions are amorphous or hyaline in their early

stages and are frequently vacuolated as they become older. They may be heterogeneous (composed of acidophilic and basophilic portions) in their composition. The shape may be spherical or otherwise symmetrical, or may be irregular. Usually the margin of the hyaline type may be joined to radiating linin strands. Again no generalization can be made about the nuclear reaction. Types A and B connote, therefore, two groupings of intranuclear inclusions on the basis of morphology only.

Herpex simplex [22, 23] and yellow fever [23] are typical examples of Type A inclusions. The cytological study of Findlay [24] would place Rift Valley fever in the same group. Poliomyelitis [25, 26, 27] and Borna disease [28] are typical examples of Type B inclusions. The submaxillary gland diseases of guinea pigs,[29] ground moles,[30] rats,[31] children,[32] and fox encephalitis also, are considered to be in Type B. In the case of the submaxillary gland diseases, however, if further study should confirm Markham's [33] opinion that the submaxillary gland disease of the guinea pig produces granular inclusions in the monocytes instead of vacuolated ones, and that the granules represent the virus particles as he has suggested, then probably all of the submaxillary gland disease group would have to be returned to Type A. Type B includes also inclusions produced by such chemicals as lead,[34] aluminum hydroxide, aluminum oxide, ferric hydroxide and carbon.[35] Probably salvarsan [36] and salyrgan [37] should be included also.

The present investigation on fox encephalitis in conjunction with the cytological study on the submaxillary gland virus of guinea pigs has given the course of development of two examples of Type B inclusions in sufficient detail so they may be compared with cytological studies which have been published on Type A inclusions. It has brought out also that Type B inclusions may be composed of two parts, a basophilic and an acidophilic substance. In the submaxillary gland disease the acidophilic substance seems to have no connection with oxychromatin, whereas in fox encephalitis it was clearly derived from oxychromatin except in the liver. The basophilic part in submaxillary gland inclusions may have derived its staining reaction from dissolved basichromatin, whereas in fox encephalitis there seems to be no apparent connection between the basophilic portion of the inclusion and the basichromatin.

This investigation has emphasized that any reconstruction of the course of events in inclusion formation must take into account and distinguish clearly those processes due to autolysis which may accompany the action of a virus upon the body or tissues generally from that entirely confined to the cell which represents the interaction between the processes of inclusion formation and the normal constituents of the nucleus. Daubney and Hudson [38] make such a distinction in Rift Valley fever but in many articles the point is ignored.

When autolysis does not interfere with the course of nuclear reaction to inclusion formation there takes place, both in submaxillary gland disease and in fox encephalitis, a return migration of basichromatin from the nuclear membrane centrally whence it applies itself to the surface of the inclusion body. The return of chromatin from the margin to the inclusion body has been recorded in human submaxillary gland disease [32] as well as in the guinea pig. The clumping of chromatin in the center of the nucleus has been observed in dying and in normal cells. Schmaus [13] observed it in the former type and Heinbecker and O'Leary,[39] Davenport, Ranson and Terwilliger [40] and Lee [41] observed it in functional nerve cells after the injection of hypertonic salt and sugar solutions. Recovery took place rapidly with no apparent damage to the cell. Buck and Boche [42] watched the process of chromatin contraction in the salivary gland cells of Sciara and likewise report that if not carried too far the cell fully recovers. These investigations would seem to suggest that when central movement of chromatin takes place one is witnessing an osmotic effect although it is difficult to visualize the mechanism. The reaction may take place in a living or a dying cell and is not in itself a cytological expression of death, but probably signifies a certain degree of cell injury from which the cell can recover if given the opportunity.

Several have considered the possibility that either the plasmosome or karyosome nucleolus becomes transformed into an inclusion body under the action of a virus, for example in yellow fever,[23] but the "straw-man" set up has always been torn down. One exception exists: Findlay [43] in his study of a disease affecting the livers of Clacton mice has found that the inclusion is a hypertrophied nucleolus. The present study has shown that in the case of fox encephalitis wherever nucleoli are present in inclu-

sion-bearing cells they always marginate with the chromatin.

Centrifugation studies have contributed the information that the oxychromatin of the inclusion body in fox encephalitis retains the same tendency to move toward the centripetal pole as does the oxychromatin when it is part of a normal nucleus, which supports the idea that they are identical materials.

Equally important is the information which centrifugation contributes to the question of what is oxychromatin, basichromatin and oxychromatic degeneration. Numerous investigators have applied the term oxychromatin and oxychromatic degeneration to the acidophilic granules which aggregate together in herpes, yellow fever, poliomyelitis and other virus diseases. It is felt that in many cases the terms have been used loosely. In the present study it has been possible to separate the two chromatins by centrifugation; the oxychromatin proves to be lighter than the basichromatin but heavier than the nuclear sap. If the inclusions of herpes represent an accumulated mass of oxychromatin, as suggested, for example by Luger and Lauda,[36] then after centrifugation it ought to lie below the nuclear sap as does the oxychromatic portion of fox encephalitis inclusions, but instead, in herpes the acidophilic inclusion proves to be lighter than anything else in the nucleus,[44] which would suggest that the inclusion body of this virus is something different from oxychromatin. Nicolau and Kopciowska [45] consider the inclusion to be composed of virus particles.

It is felt that the use of the ultracentrifuge offers a means for more complete analysis and identification of component parts of inclusion bodies and the cells which contain them than has been available previously and that the instrument should be used more extensively than it is now.

NOTE: I am grateful to the Research Council of Iowa State College for subscribing to a room at the Marine Biological Laboratory where part of this work was done and for granting funds to pay part of the cost of the colored plate.

SUMMARY AND CONCLUSIONS

1. Intranuclear inclusions produced by fox encephalitis virus have been studied in order to arrange, as far as possible, the sequence of cytological changes caused by the disease. Most of

the data are obtained from cells of the lamina epithelialis of the tela choroidea.

2. Two types of reactions are distinguished; the cytological changes which accompany autolysis, and the reactions of the nucleus to the presence of an inclusion body. It is concluded that the two are separate processes.

3. Autolysis in the nucleus of non-inclusion-bearing cells is first revealed by an abnormal increase of chromatin granules. This is followed by coalescence of the granules and dissolution of some of the material so that the nuclear sap becomes Feulgen-positive, and usually shrinkage of the nucleus accompanies these changes. The final condition is a pyknotic nucleus, in which the contents are vacuolated but otherwise homogeneous.

4. The sequence of nuclear changes produced by the inclusion body is based on the degree of reaction of the nucleus rather than on the size of the inclusion body.

(*a*) The first nuclear reaction is a pulling away of linin network and chromatin granules from the inclusion body which eventually results in the formation of a "halo." The inclusion is Feulgen-positive from its earliest recognizable beginning.

(*b*) During the process of margination the basichromatin and oxychromatin separate from each other and move in opposite directions; the basichromatin continues toward the nuclear membrane and the oxychromatin returns to the inclusion body which it encloses as an acidophilic mantle.

(*c*) The original basophilic center of the inclusion and the oxychromatin mantle mingle to varying degrees and among different cells the two substances are present in different proportions. On this basis are discussed the various tinctorial effects obtained and it is concluded that acidophilic and basophilic inclusions are fundamentally alike in fox encephalitis.

(*d*) The basichromatin, after complete margination, tends to aggregate into clumps. Subsequently these migrate inward from the nuclear membrane and become adherent to the surface of the inclusion. The "halo" persists but is displaced outward.

5. Autolysis may stop the nuclear reactions at any point in the series of events which accompany inclusion formation. Its most conspicuous effect is shrinkage of the nuclear membrane.

6. The reactions in the ependymal cells are essentially the

same as in the lamina epithelialis. Endothelial cells with inclusions were too few to obtain a complete record but those studied seemed to correspond to stages described for cells of the lamina epithelialis. Liver cells with inclusions were few also, but it was evident that the inclusion is different in that the oxychromatin does not join the inclusion body.

7. Oxychromatin and basichromatin of the normal nucleus in cells of the lamina epithelialis and liver have been completely separated by centrifugation. The oxychromatin forms a homogeneous layer on the centripetal side of the basichromatin and has, therefore, a specific gravity less than basichromatin but greater than nuclear sap.

8. Centrifugation of inclusion-bearing cells in the lamina epithelialis causes the marginated chromatin to move toward the centrifugal pole; the inclusion body comes to lie centripetal to it and in many cases the oxychromatin portion of the inclusion tends to be concentrated on the centripetal side of the basophilic center. Centrifugation of liver cells which contain inclusions gives about the same reaction, except that the inclusion body lacks an oxychromatin portion.

9. The use of the centrifuge in the identification of oxychromatin and basichromatin and its bearing on oxychromatic degeneration is discussed. It is concluded that the intranuclear inclusion of herpes simplex is not oxychromatin.

10. The knowledge obtained from several virus studies has enabled a more exact statement to be made for the identification and classification of Cowdry's Types A and B intranuclear inclusions.

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DESCRIPTION OF PLATE

Drawings were made with a camera lucida, and have a magnification of × 3200. Nuclei shown in Figures 1–12 were fixed in corrosive-acetic and stained with Feulgen's reagent and light green; those in Figures 13–23 were fixed in Zenker's with acetic and stained in azure-eosin. The nuclei shown in Figures 19–23 were centrifuged at forces between 300,000 and 550,000 times gravity and are arranged on the page so that the centrifugal pole is toward the bottom of the plate. Figures 1–21 are of nuclei from the lamina epithelialis and Figures 22 and 23 are of liver cell nuclei.

PLATE 129

FIG. 1. A normal resting nucleus which shows Feulgen-positive chromatin granules uniformly distributed over the linin network.

FIG. 2. A nucleus which in the early stages of autolysis shows an abnormal increase in chromatin.

FIG. 3. The chromatin granules have coalesced and chromatin has gone into solution so that the nuclear sap stains with Feulgen's reagent. There has been some shrinkage.

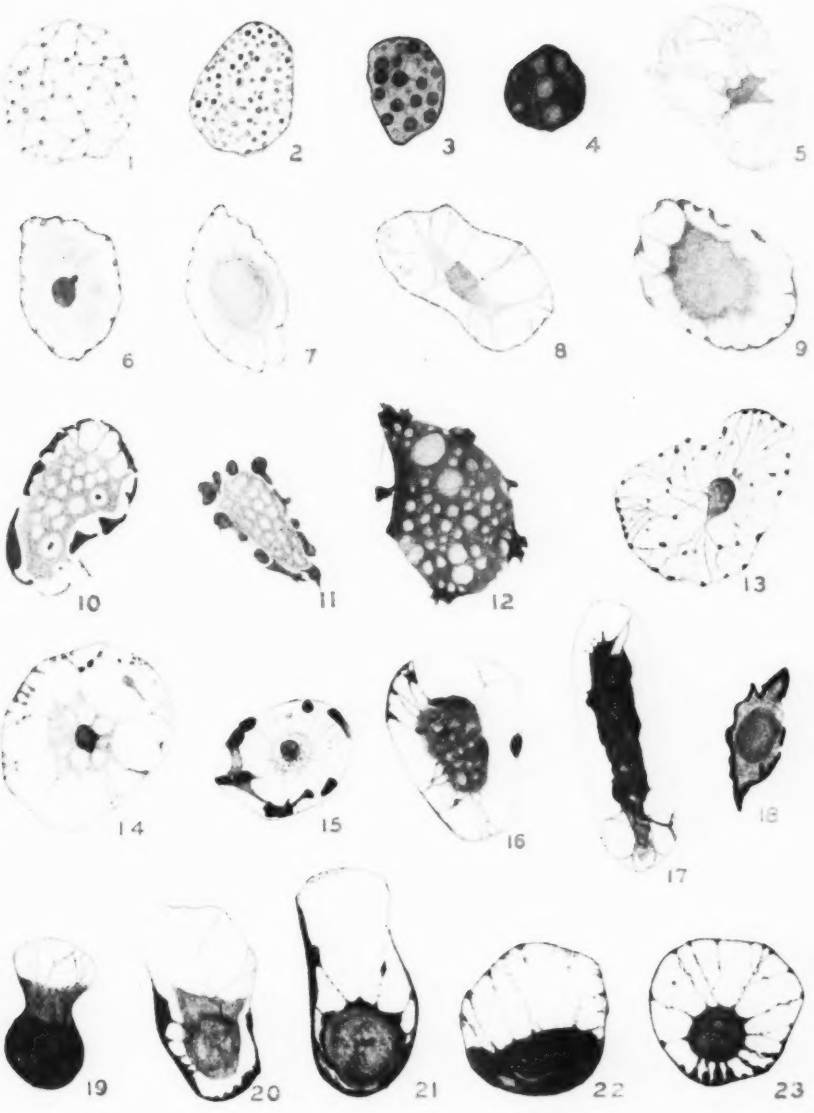
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FIG. 4. A pyknotic nucleus which shows shrinkage of the membrane and a homogeneous interior except for vacuoles.

FIG. 5. An early stage in inclusion formation. The inclusion is Feulgen-positive. The chromatin granules and linin network have begun to retract toward the margin.

FIG. 6. The basichromatin and oxychromatin have separated. Most of the former has reached the nuclear margin; the latter, colored green, is approaching the basophilic inclusion.

FIG. 7. The basichromatin is completely marginated and the green staining oxychromatin has surrounded the inclusion body.

FIG. 8. The marginated basichromatin shows the beginning of a reaction to aggregate into clumps. The arrangement of oxychromatin as shown in this figure was observed frequently.

FIG. 9. There is further clumping of basichromatin into irregular masses. The basophilic inclusion and oxychromatin have mingled to a considerable degree.

FIG. 10. The basichromatin masses are large and more irregular than in Figure 9. Some shrinkage has occurred. Of the two spheres of basichromatin in the inclusion, one can be traced, by focusing, to the margin and the other is apparently detached from other chromatin. The inclusion stains uniformly except for the presence of vacuoles which are characteristic of ageing.

FIG. 11. The basichromatin masses have rounded and by the shrinkage of the nuclear membrane, characteristic of autolysis, they are brought against the inclusion body.

FIG. 12. A particularly large inclusion body with numerous vacuoles. The amount of basichromatin is probably about the same as in Figure 11 but due to the large size of the inclusion the masses are farther apart.

FIG. 13. A stage nearly identical with Figure 5. With azure-eosin the Feulgen-positive inclusion stains with the basic dye.

FIG. 14. This stage is slightly more advanced than Figure 6 in that the oxychromatin has joined the inclusion body.

FIG. 15. The clumped basichromatin is pulling away from its position of complete margination and has started to move in the direction of the inclusion. The basophilic center and the mantle of oxychromatin may be distinguished in the inclusion.

FIG. 16. About half of the originally marginated basichromatin has returned to the inclusion body. It is probable that autolysis has begun also.

FIG. 17. All of the originally marginated chromatin has returned to the surface of the inclusion body. The halo has thereby been displaced outward to lie between basichromatin and nuclear membrane. The opacity in staining probably indicates that autolysis has begun its action on this cell but it has not reached the stage which produces shrinkage of the nuclear membrane.

FIG. 18. Autolysis produced its effects upon this nucleus when it was probably in a stage about like Figure 7 or 9. Death reaction set in before the basichromatin had migrated from the nuclear membrane to the inclusion body.

FIG. 19. A non-inclusion-bearing nucleus which has been centrifuged. The heavier basichromatin is concentrated at the centrifugal pole and the oxychromatin has been pressed out from the basichromatin to form a layer between the nuclear sap and basichromatin.

FIG. 20. An inclusion-bearing nucleus. The basichromatin has not been concentrated as completely as in Figure 19. The oxychromatin portion of the inclusion tends to move to the centripetal side of the basophilic portion.

FIG. 21. An inclusion-bearing nucleus in which part of the basichromatin has returned from the margin and joined the inclusion body. Centrifugation fails to separate them.

FIG. 22. A nucleus of a liver cell. When centrifuged the inclusion comes to rest centripetal to the chromatin.

FIG. 23. Centrifugation moved the inclusion only slightly. Some of the chromatin has been concentrated at the centrifugal pole.

ETIOLOGY OF CALCIFIED NODULAR AORTIC STENOSIS *

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Two ideas are generally accepted relative to the etiology of nodular calcified aortic stenosis. According to one idea many of these lesions are the result of rheumatic infection; according to the other, in the older age groups at least, the lesion is degenerative or arteriosclerotic in origin. Most investigators hold that both theories are operative. Recently, however, Clawson, Noble and Lufkin [1] have presented evidence favoring a rheumatic etiology in all cases. Christian,[2] who formerly believed in a divided etiology, now considers a rheumatic genesis highly probable in all cases. He arrived at this conclusion mainly on clinical grounds. Dry and Willius [3] published in 1939 a clinical study of 228 cases of calcareous aortic stenosis. They state: "The study of this material has led us to accept, unequivocally, rheumatic infection as the etiologic factor in calcareous stenosis of the aortic valve." The authors believe that the rheumatic infection which eventually culminates in aortic stenosis is originally a mild form of rheumatic carditis which affects chiefly the aortic valve.

In approximately 40–45 per cent of cases of calcified aortic stenosis there is an associated lesion of the mitral valve. Invariably other stigmas of rheumatic infection are present as well, so that the etiology in these cases is quite evident. The remaining 50–55 per cent of patients with aortic stenosis have the aortic valve alone affected. These patients tend to survive longer than those with a combined mitral and aortic lesion. Clawson and his associates found that patients with an aortic lesion died 15–20 years later on the average than those with a combined mitral and aortic lesion. Hence, many patients with a single aortic lesion will live to the sixth or seventh decade. A few survive even to the eighth decade. This group of patients, therefore, reaches the age period in which general arteriosclerosis is most common.

In spite of this fact, the aorta even in elderly patients is usu-

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ally quite smooth in the presence of aortic stenosis. Apparently the reduced intra-aortic pressure tends to spare the aorta much wear and tear in these cases. Nevertheless, during the past 40 years there have been many investigators who have believed that the calcified aortic valve is directly related to the atheromatous process in the aorta. The general idea of a degenerative or arteriosclerotic process as the basis for calcific aortic stenosis is ascribed to Mönckeberg.[4] He published in 1904 a careful description of the normal histology of the aortic valve, together with the microscopic anatomy of the sclerosed valve. Mönckeberg spoke of an *ascending* sclerosis which began in small atheromatous areas within the sinuses of Valsalva, especially along the "insertion margins" of the cusps. In this group the aorta was smooth. Beginning in the angle of insertion the degenerative process progressed along the inner or fibrosa layer of the cusps, ascending to the closing edges. The fibrous thickening and calcification which resulted was found to be most marked in the bases of the sinuses near the insertion margins. The wear and tear resulting from the force of the blood column rebounding on the cusps following systole was believed to be responsible for the changes described. Mönckeberg also described a *descending* type of sclerosis which was thought to originate in the atheromatous lesions of the aorta and pass by way of the commissural attachments to the closing margins of the cusps. This type of sclerosis seems to be confused with syphilis of the aortic valve and perhaps also with rheumatic endocarditis. The general idea of the calcific aortic valve arising directly from the arteriosclerotic process in the aorta has been credited to Mönckeberg under the caption of "Mönckeberg's aortic sclerosis."

Among the earlier workers who accepted the arteriosclerotic genesis of aortic valve sclerosis may be mentioned Birch-Hirschfeld, Kaufmann and Schmaus (quoted from Mönckeberg [4]).

Among the more recent workers who agree that Mönckeberg's aortic sclerosis is operative in calcific valve disease may be mentioned Libman [5] and Sohval and Gross.[6] In 1936 Sohval and Gross [6] reported a series of 50 hearts showing nodular aortic stenosis. After careful gross and microscopic examination they classified these etiologically as follows: 18 cases of Mönckeberg's aortic sclerosis, 19 cases of polyvalvular extinct rheumatic, and

13 cases of monovalvular extinct rheumatic sclerosis. In the 18 cases showing Mönckeberg's type of sclerosis they failed to find distinctive rheumatic stigmas. The authors concluded that these cases are purely degenerative. Furthermore, they state that these cases are readily separated from those of rheumatic origin on microscopic grounds.

Anitschkow [7] classifies the lesions of the heart valves (aside from syphilis) under: (*a*) atherosclerosis (lipoidosis), (*b*) inflammatory changes, and (*c*) combined inflammatory and atherosclerotic changes. He states, however, that primary atherosclerosis leads neither to the development of cicatricial tissue nor to deformation and ulceration of the valves, and therefore has no great independent importance in the origin of heart valve defects.

On the other hand, Clawson, Noble and Lufkin [1] in 1938 reported a series of 200 cases of calcified nodular deformity of the aortic valve. These were studied from the standpoint of the clinical characteristics, frequency of occurrence, etiology and pathogenesis. A history of rheumatism was found in 35 per cent of their patients, and an adherent pericardium in 16 per cent. The association of a mitral or other valve deformity with the calcified aortic lesion was present in 44.5 per cent of the 200 cases. On microscopic examination Aschoff bodies were found in 13 per cent of both the aortic and the polyvalvular groups. Cholesterol crystals commonly found in atherosclerosis were not seen in any of the sclerotic valves. Following this careful investigation Clawson *et al.*[1] conclude in part as follows: "The calcified nodular aortic valve deformity is most probably the result of repeated attacks of rheumatic proliferative inflammation with calcification similar to that commonly seen in the mitral leaflets."

Material and Methods

The 31 unselected cases here presented have been studied largely from the morphological standpoint. The clinical records were consulted for the age, sex and possible history of rheumatic infection. The hearts were described minutely in the gross. All of them were examined microscopically. Those with polyvalvular lesions and hence of rheumatic etiology served as controls in establishing rheumatic stigmas of importance. We have had

difficulty in finding adult hearts with strictly normal valves. We have preferred, therefore, to use the known rheumatic hearts as controls. In general, six standard blocks were taken from each heart after the method of Gross, Antopol and Sacks.[8] In a few of the hearts which were definitely rheumatic only 2–3 blocks were studied. Tissues were embedded in paraffin and stained with hematoxylin and eosin and Weigert's elastic stain. The latter was at times combined with the Van Gieson method.

The Gross Appearance

The typical advanced lesion of calcified aortic stenosis has a characteristic gross appearance. There is fibrous thickening with calcification of the closing edges of the cusps, which causes them to stand out rigidly. The commissures of the cusps are fused often for a distance of 0.5 to 1.5 cm. and may be 0.5 cm. or more in thickness and thoroughly calcified. Irregular calcified nodules are frequently seen within the sinuses of Valsalva, at times almost completely filling them. On the ventricular side buttresses of calcific material commonly reinforce the thickened cusps at their bases. The above changes (Fig. 1) reduce the orifice of the valve to a considerable degree, depending upon the extent of the process. In several cases a mere slit-like opening, perhaps 6–8 mm. long by 1 or 2 mm. in width, affords the only passage between the left ventricle and aorta (Fig. 2). These hearts are very large, due to an extreme work hypertrophy of the left ventricle. Lesions of this type with heavy fused commissures have in our experience proved to be invariably rheumatic in origin. The aorta is usually smooth, especially if the stenosis is severe. The blood pressure is commonly low in the aorta; thus wear and tear are greatly reduced. In practically all of the hearts in which the aortic orifice is reduced to a narrow slit an "impingement" plaque is found (Fig. 3). This is an early, fatty, slightly elevated atheromatous lesion often 6–8 cm. long and usually only 1–1.5 cm. wide. It is located, in our material, most frequently on the median or posterior side of the ascending aorta. It begins a short distance above the orifice and extends often to the beginning of the arch. We consider this lesion the result of impingement of a narrow stream of blood of great velocity against the intima of the aorta. Although the blood pressure is low in the aorta because of reduced volume of blood,

the rate of flow through these slit-like openings must be very great, impelled as it is by a powerful left ventricle.

Four hearts in our series showed calcified nodular masses within the sinuses of Valsalva without much thickening of the free edges of the cusps. Smaller calcified masses occur in some instances at the bases of the cusps on the ventricular side. There may be no fusion of the commissures and only moderate stenosis of the aortic orifice. Hearts of this type are usually small, weighing only about 400 gm. The rheumatic process is not so evident in such a picture, yet of the 4 cases of this kind all but 1 seem to be definitely of rheumatic origin. The 4th case has several stigmas of probable rheumatic character. The mild degree of atherosclerosis of the aorta is against its acceptance in the group of Mönckeberg's sclerosis.

A modification of the gross lesion was found in 29 per cent of our cases due to the presence of a bicuspid aortic valve in which one of the commissures, most frequently the left-right, was dwarfed. A low calcified ridge or raphe represents the line of fusion of the two cusps (Fig. 4). According to Gross [9] the bicuspid aortic valve in adults was associated with rheumatic infection in 14 out of 16 cases which he studied. If no congenital anomaly is found in the heart associated with a bicuspid valve he believes the latter should be considered an acquired lesion. Among the 8 hearts with a bicuspid aortic valve no congenital lesion was found.

Abbott [10] speaks of the almost constant presence of secondary inflammatory changes in bicuspid aortic valves which makes it difficult to decide whether or not a given lesion is a congenital or acquired one. Even valves of congenital origin have a high incidence of rheumatic involvement.

Histological Examination

The histological changes in the heart in acute rheumatic infections are well established by the work of numerous investigators in this field. The microscopic changes in the chronic forms of the disease are less striking, are more difficult of interpretation, and are not so well known. It will be necessary, therefore, in describing the histopathology to refer frequently to the literature and to compare our findings from time to time with lesions seen in the

more acute phases of rheumatic infections. In this way criteria may be established for the recognition of the various stigmas of rheumatic fever as they occur in the so-called "healed" stages of the disease.

The Aschoff Body: This is the most reliable sign of rheumatic infection in the chronic as well as in the more acute stages of the disease. Although Clawson [11] in his review of the subject does not regard the Aschoff body as absolutely specific for rheumatic infection, yet Gross and Ehrlich [12] as well as others, do so regard it. It is customary to think of the Aschoff nodule as occurring mainly in connection with acute or subacute rheumatic processes. It is surprising how frequently this phenomenon is seen in the myocardium of the so-called healed cases of rheumatic disease. Clawson and his associates [1] found Aschoff nodules in only 13 per cent of their polyvalvular and 13 per cent of their monovalvular "healed" lesions. Gross, Antopol and Sacks,[8] using their standard method for removing tissue for sectioning, described Aschoff bodies in 42 cases out of 79 of acute and chronic rheumatic infection, an incidence of 53 per cent. Gross and Ehrlich [13] in a study of 161 cases of active and healed lesions found Aschoff bodies present in 59 per cent.

Use of the standardized method referred to above [8] requires that blocks be taken from each of the valves, including adjoining structures such as aorta, pulmonary artery and adjacent myocardium. Blocks are also taken from the posterior papillary muscle and from the wall of the left auricle. Material obtained in this way has yielded structures which we have interpreted as Aschoff bodies in 58.3 per cent of our polyvalvular cases and 47.3 per cent of our monovalvular cases. These figures seem rather high for material from the chronic stages of the disease, but correspond quite closely with the statistics of Gross and his associates [8, 13] cited above.

We are fully aware that considerable difference of opinion exists regarding the recognition of Aschoff nodules. Some authors insist on a strict conformity to the classical description. This is to be expected of the earlier writers on the subject; however, some recent investigators (Saphir and Wile [14]) also hold to this idea. This is well and proper if one is dealing only with active cases of rheumatic infection. Aschoff and Tawara,[15] Coombs,[16]

and other early writers on the subject were dealing largely with cases of active or recent infection. Geipel [17] was one of the first observers to suggest that Aschoff bodies pass through a sort of life cycle. He stated that the ground substance eventually becomes fibrillar and transforms itself into connective tissue. Klinge,[18] in his monograph on rheumatism published in 1933, describes clearly a series of stages in the development of the Aschoff nodule. In the early stages hyaline masses of swollen collagen and fibrin are seen interspersed by large cells with a basophilic cytoplasm. Usually multinucleated giant cells are present in addition to smaller cells of mesenchymal origin. Later, through involuntary changes the giant cells, fibrin and hyaline matrix disappear to be replaced by scar tissue, which is more or less cellular.

In 1934 Gross and Ehrlich [12] described several types of Aschoff bodies which they believed represented different stages in the life cycle of the lesion. In the same year the authors published studies in which they classified Aschoff bodies into seven different types. We believe with Klinge, Gross and Ehrlich, and others, that the Aschoff body undergoes a series of changes or transformations, especially in individuals in whom the rheumatic process persists for many years. No doubt new granulomas arise with exacerbations of the disease. These in the course of weeks or months pass through a series of cyclic changes resulting finally in scars within the myocardium, which may be more or less cellular. Numerous small scars relatively rich in pale oval or elongated nuclei are seen in the heart muscle in our material.

Careful study of the 9 cases in which Aschoff bodies were found in our monovalvular group shows instances of the reticular, the small celled coronal, and the polarized and fibrillar types. A characteristic small celled, partly polarized Aschoff body is seen in Figure 5. This is composed of small mesenchymal cells, oval to moderately elongated. An occasional larger cell is seen having fairly abundant cytoplasm. In Figure 6 we see a fibrillar polarized type. In 3 of our cases (689, 21176 and S–3375) there was an acute terminal myocarditis of focal character. In 2 of these miliary abscesses appear in the heart muscle. Figure 7 is from one of these cases (21176). It shows hemorrhage into the myocardium and an area of cellular infiltration predominantly of large mononuclear cells with a few polymorphonuclears present.

The Aschoff bodies in our cases are composed largely of small mesenchymal cells among which may often be found a few larger cells with fibrocytoid and "owl-eyed" nuclei. Occasional multinucleated cells are present. It is unusual to find a central necrotic mass of collagen such as may be commonly seen in the acute cases.

Chronic Inflammation at the Roots of the Aortic Cusps: Microscopic sections of the aortic valve were made in 9 of the 12 polyvalvular cases. About 50 per cent of these revealed moderate to fairly well marked inflammation about the root of the valve. Three of the 9 cases exhibited but slight reaction. Eleven of the 19 monovalvular cases presented similarly a definite to a well marked chronic inflammation in this group between the ages of 65 and 79 years (Fig. 8). One patient had a moderate degree while two others had but slight inflammatory changes in the valve attachment. The two oldest patients in the monovalvular group, each 81 years, presented slight to moderate inflammation at the valve root. If the entire aortic valve could be serially sectioned the percentage showing chronic inflammation would no doubt be considerably higher. Six sections cut from one block at 10 μ each would constitute not more than 1/600 of the circumference of the aortic ring, assuming the latter to average 4 cm. in its stenosed condition. It stands to reason that the percentages of chronic inflammation in the two groups would increase markedly if the complete ring were examined.

In a number of instances ossification with formation of marrow spaces was seen in the calcified areas. This was most pronounced in Case 3650 (Fig. 9).

Regularly the inflammation occurs at the roots of the cusps, often in proximity to calcific material. Only occasionally are small infiltrates of cells seen about the calcium deposits elsewhere. It would seem to be a reaction to some organism or toxin and not a foreign body response. That it represents a reaction to rheumatic infection seems logical. One must assume then that the rheumatic virus remains in the tissues for many, many years, or that recurrent attacks occur from time to time. Perhaps these are in the nature of allergic reactions which are induced from time to time by upper respiratory infections. The presence of Aschoff bodies, inflammation and necrosis of vessel walls, and other cardiac rheumatic stigmas found in patients even in their sixth or seventh

decade, afford further evidence of a smoldering or recurrent infection.

Thickening of Endocardium: Irregular thickening of the endocardium, especially of the left auricle, with condensations, reduplications and thinning of the elastic fibers, are criteria we have found present in practically all of our cases. These changes were first described by MacCallum [19] in 1925. Increased capillarization and mild round cell infiltration of the subendothelium of the left auricle are likewise considered stigmas of rheumatic infection. Mild fibrous thickening of the mitral, tricuspid, and occasionally of the pulmonic leaflets may show on microscopic examination an irregular thickening with a myxomatous connective tissue reaction (Fig. 10). These changes, especially if accompanied by increased numbers of plump, fusiform mesenchymal cells, are similar to those seen in the more active forms of rheumatic endocarditis (Chiari [20]).

Pericarditis: Old adhesive pericarditis is most frequently the result of a rheumatic process. Microscopic pericarditis and nests of cells of the Aschoff type in the subepicardium are similarly reliable indications of rheumatic involvement. Forty-eight per cent of our combined cases presented adhesive pericarditis of some degree. Of the polyvalvular cases 66.6 per cent, and of the monovalvular cases 36.8 per cent showed some degree of pericarditis.

Vascular and Perivascular Rheumatic Lesions: Karsner and Bayless [21] have investigated in a comprehensive manner the rheumatic lesions occurring in the coronary arteries. Von Glahn and Pappenheimer [22] have described the characteristic lesions of rheumatic infection in the vascular and perivascular tissues of the myocardium. It is in the branches of the coronary arteries and their perivascular spaces that some of the most important lesions of rheumatic fever are manifest. Von Glahn and Pappenheimer [22] speak of swollen endothelium which is basophilic in staining. The wall of the vessel is thick as compared with the caliber of the lumen. Swelling in the early phases is due to infiltration of the walls with fibrin. Klinge [18] refers to this as "fibrinoid Verquellung" and the accompanying necrosis he calls "fibrinoid necrosis." These terms are in general use by the German writers. An example of fibrinoid swelling is seen in the

wall of the artery and perivascular spaces in Case S–2363 (Fig. 11). In acute rheumatic fever this material takes the Weigert fibrin stain, stains yellow with the Van Gieson method, and the fibrils may be impregnated with silver after the method of von Pap (Chiari [20]). In the chronic cases of rheumatic heart disease a fine, almost hyaline fibrillar substance usually devoid of nuclei is found about the coronary arterioles in many instances. Optically the material is quite similar to the fibrinoid swelling of the more active cases but shows evidence of collagenous change. The staining reactions have also changed. Fibrin can only occasionally be demonstrated by the Weigert method, with the Van Gieson stain the scar-like masses stain faintly red, and with Mallory's connective tissue stain blue fibrils are conspicuous. We interpret these perivascular scars as healed phases of the earlier fibrinoid reaction. Von Glahn and Pappenheimer [22] state that the fibrinous exudate is gradually changed to a permanent tissue. Klinge [18] regards these scars as typical of the chronic phase of rheumatic heart disease. The scars are of an oval or spindle form and appear conspicuously about the vessels. These are especially abundant in sections of the posterior papillary muscle.

Hyaline acellular areas are seen also in many of the small arteries of the heart. At times these are swollen and more deeply eosinophilic than the remainder of the vessel wall.

Another change commonly seen in the arterial wall is a peculiar disorganization of the media. Smooth muscle fibers run in various directions, muscle nuclei are swollen or pyknotic, and wandering cells in small numbers may be present. These changes are very prominent in healed polyvalvular rheumatic heart disease. In some of the younger patients this condition is similar to the "arteritis rheumatica" described in acute rheumatic infections (Fig. 12) by Chiari [20] and others.

Several cases in our series show what Karsner and Bayless [21] designate as "giant medial hypertrophy," a lesion which is frequently seen in active cases of rheumatic infection. The smooth muscle of the media of the small arteries is greatly hypertrophied and often more or less disorganized (Fig. 13). Another condition described by the same authors is caused by edema of the media. A loose reticulated zone develops within the muscle known as "état reticulaire." One of cour cases presents such a picture in

some of the arterioles of the adventitia of the aorta and near the aortic root (Fig. 14).

Mononuclear Cell Infiltration: In addition to Aschoff bodies, which have already been discussed, groups of mononuclear cells are found infiltrating the perivascular and interstitial spaces to varying degrees. The cells vary from small lymphocytes to large mononuclears, many of which appear to be mesenchymal wandering cells. Polymorphonuclear neutrophils are also present in a few cases. We regard such infiltrations as strong presumptive evidence of rheumatic involvement, especially when accompanied by other lesions of rheumatic taint.

While the more important stigmas left by the rheumatic infection have already been outlined, there are some changes in the muscle proper which should be mentioned. Aschoff bodies are occasionally seen within the muscle. Edema with marked separation of the muscle bundles is regularly seen in the wall of the left auricle and to a less extent in the ventricles.

Scarring of Myocardium: Interstitial infiltration of large mononuclears and plump fusiform mesenchymal cells is one of the commonest characteristics suggesting rheumatic involvement. Interfascicular scars are considered of importance by a number of authors. We have observed extensive cellular scarring not confined to the interfascicular areas in some of our cases. The most extreme case of this kind is seen in Case 17455, a young woman of 20 years. The aorta shows one or two early atheromatous plaques, apparently of the "impingement" type. The first part of the left coronary is thickened for a short distance, but this appears to be adventitial rather than intimal. Microscopically prominent cellular scars are found in the wall of the left ventricle and especially in the posterior papillary muscle (Fig. 16). Microscopic sections of the left coronary and its main branches show thin walls with slightly dilated lumens.* In the absence of atherosclerotic lesions it would seem best to consider the scarring a part

* Blocks from the left coronary artery and the proximal portions of the descending and circumflex branches were sectioned and studied. All showed unobstructed lumens with thin arterial walls. The intima in all sections was uniformly thickened, averaging about the same as the media. The latter appeared to be considerably thinned since the whole wall was of less than normal thickness. The intimal proliferation consists of connective tissue which is somewhat loosely arranged in places. The internal elastic membrane is of normal thickness, but traverses the midportion of the wall.

of the rheumatic picture in this 20 year old girl. Rheumatic arteritis which is conspicuously present in many of the smaller coronary arteries (Fig. 12) is probably a factor. Due to swelling of the wall and infiltration of wandering cells many arterioles are seriously damaged if not actually obstructed. Perivascular scarring has already been considered.

Two tables have been prepared in order to present the data of the various cases in concise form. In Table I all of the 31 cases are included; 12 cases with mitral and aortic valve lesions, 19 with aortic valvular lesions only. The clinical data include race, age, sex and history of any previous rheumatic infection. The anatomical data consist of the weight of the heart, grade of aortic and mitral lesions, and the presence or absence of the following: Aschoff bodies, inflammatory changes at the root of the aortic cusp, other changes which may be considered probable rheumatic stigmas, arteriosclerosis of the aorta and coronary arteries, and the incidence of bicuspid aortic valve. The final column contains pertinent comment and additional data.

Analysis of Data, Table I

All patients belonged to the Caucasian race; however, there are two Mexicans 53 and 72 years of age, respectively. Ages ranged from 20 to 86 years. The average age for those patients with both mitral and aortic lesions was 59.3 years. The average weight of the heart in the patients with a double lesion was 618 gm.; in those with the aortic lesion only, the average weight was 571 gm.

The following data refer to the group of 12 patients with a *double valvular lesion:*

There were 9 males and 3 females, 75 and 25 per cent, respectively. Seven patients had a history of previous rheumatic infection, or 58.3 per cent. There was a negative history in 2 patients (16.6 per cent), and in 3 patients (25 per cent) no data were recorded. In 7 patients (58.3 per cent) Aschoff bodies were found; none were seen in 5 (41.7 per cent). Atherosclerosis of the aorta was absent in 3 cases, or 25 per cent; slight (+) in 6 cases, or 50 per cent; and moderate in 3 (++) cases, or 25 per cent. This is a distinctly low grade of arteriosclerosis, considering the average age of 59.3 years.

Adhesive pericarditis was present to some degree, often slight, in 9 patients, or 75 per cent.

Nineteen patients with a solitary *aortic lesion* present the following comparative figures:

There were 15 males (78.9 per cent) and 4 females, or 21 per cent. History of previous rheumatic infection was obtained in 8 instances, or 42.1 per cent; history was negative in 7 (36.9 per cent); while no data were recorded in 4 cases (21 per cent). Aschoff bodies were discovered in 9 hearts, or 47.4 per cent. No atherosclerosis of the aorta was observed in 2 cases (10.5 per cent); it was of slight degree (+) in 11, or 57.9 per cent; moderate (++) and marked (+++) were recorded each in 3 cases, or 15.8 per cent. In spite of an average age of 62.7 years, marked degenerative changes were found in the aortas of only 3 patients out of 19. Furthermore, the figure corresponds in general with the usual low incidence of arteriosclerosis in rheumatic heart disease. Stenosis of the aortic valves was not severe in any of the hearts in which arteriosclerosis of the aorta was marked. The above data support the idea that the aorta is generally smooth in cases of aortic stenosis.

Analysis of Data, Table II

Since we are most concerned with the group in whom the aortic valve alone is involved, Table II has been assembled to provide a more detailed analysis of these particular cases. In this table an attempt is made to present a quantitative estimate of the pathological changes in several features, *viz.* the degree of sclerosis of the aortic valve, the amount of aortic arteriosclerosis and the more important rheumatic stigmas found in each heart of the monovalvular group. In addition are included the age of the patient, weight of the heart, and a final column for remarks. The rheumatic manifestations are classified according to the site of occurrence.

Under the heading of *aortic valve* are included calcification, aortic arteriosclerosis and the vascular and cellular inflammatory changes found near the root of the valve. Under the caption "*vascular changes*" are included the arterial lesions, such as "arteritis rheumatica," "fibrinoid" necrosis and hyperplasia of endothelium and media. The *perivascular* changes include Aschoff

TABLE I

Comparative Data on Polyvalvular and Monovalvular Cases

Case number	Race	Sex	Age	History of rheumatic infection	Heart weight	Involvement		Aschoff nodules	Inflammation aortic valve	Pericarditis	Other rheumatic changes	Arteriosclerosis		Biscuspid aortic valve	Remarks
						Aortic valve	Mitral valve					Aorta	Coronary arteries		
			yrs.		*gm.*										
21137	W	F	38	+	660	+++	+++	+	+	+	+	o	±	o	Fleshy vegetation on mitral valve; heart trouble since 21 yrs. of age; adhesions at base of heart
S-3633	W	F	40	o	880	+++	++	+	+	+	+	+	±	o	Pleurisy at 9 yrs.; few fibrous adhesions left auricle
20646	W	M	42	+	770	++	+++	+	−	+++	+	o	o	o	Marked adhesive pericarditis; old thrombus in left auricle; recurrent rheumatic fever at 12, 15, 16 yrs.
H.M.H.	W	M	44	+	700	++++	++	+	+	+	+	+	+	o	Slight pericardial adhesions; same Dr. treated rheumatic fever at 6 yrs. who treated patient in final illness
21223	W	M	46	N.R.	375	+++	+++	o	+	+	+	+	++	o	Adhesions to pericardium at base
S-3615	W	M	51	+	1050	+++	++	+	−	+	+	+	±	o	St. Vitus at 8 yrs.; inflammatory rheumatism ankles and knees 4 yrs. before death; filmy adhesions to pericardium
S-2950	W	M	56	+	580	++++	++	o	+	+	+	+	++	o	Adhesions at apex of heart; endocardial sclerosis left ventricle; rheumatic fever in 1907.
46	W	M	56	+	715	++++	+++	o	o	±	+	++	+	o	History of rheumatic fever at 12 yrs.; "soldier spots" in pericardium
21246	W	M	57	+	450	++++	++++	+	+	+	+	+	++	o	Slight roughening and opacity of pericardium; rheumatic fever at 27 yrs.
21575	W	M	70	o	500	+++	++	o	+	o	+	o	+	o	Malaria at 30 yrs. of age
19384	W	F	76	N.R.	380	+++	+++	+	+	+	+	++	+++	o	Entered in coma; adhesion of apex of heart
21224	W	M	86	N.R.	560	++	++	o	+	o	+	++	+++	o	Unconscious on entry; much epicardial fat; base of aorta 10 cm. in circumference
17455	W	F	20	o	550	+++	±	+	+	o	+	+	+	+	
39	W	M	40	o	634	+++	±	o	+	+	+	+	±	+	No history found; "soldier spots" in pericardium

TABLE I (*Continued*)

S–2363	W	M	50	+	650	++++	o	o	+	±	+	±	+	o	Slight adhesions near apex
19037	Mexican	M	53	N.R.	850	++	±	+	±	o	+	+	±	+	
689	W	M	58	o	500	++	±	+	+	+	+	++	+++	o	Pericardial adhesions at base
14868	W	F	60	+	650	+++	o	+	±	+	+	o	o	o	Pericarditis, rheumatic (microscopic)
691	W	F	60	o	560	+++	±	o	+	o	+	+	±	o	Measles twice (rheumatism?)
S–1137	W	M	61	+	775	+++	±	o	+	±	+	+	o	+	Hospitalized in 1921; malaise, chills and fever (3 wks.); tonsillitis, enlarged heart and systolic murmur
1410	W	M	62	+	410	+++	±	+	+	+	+	+	+++	o	Tonsillitis and acute arthritis (1917–18); pericardial adhesions; "impingement" plaque, aorta
2450	W	M	65	+	450	+++	±	o	+	+	+	+++	+++	±	Rheumatism, 20 years ago; few pericardial adhesions
21176	W	M	66	N.R.	450	+++	±	+	+	o	+	+	++	+	Rheumatic aortitis; miliary abscess in heart muscle
S–1474	W	M	66	o	585	++++	o	+	+	o	+	++	++	+	
3650	W	M	72	+	650	+++	±	+	+	o	+	+++	++	+	Acute rheumatism during Spanish-American war; sore throat with joint pains
19883	Mexican	M	72	N.R.	400	++	±	o	+	o	+	++	++	o	Calcified nodules in sinuses of Valsalva; slight fusion of commissures
18905	W	M	72	N.R.	525	+++	–	o	o	o	+	+++	–	+	
14390	W	F	74	o	510	+++	±	o	+	o	+	+	+	o	Rheumatism right leg 20 yrs. previously; nodular calcified masses, sinuses of Valsalva
4725	W	M	79	+	725	++++	+	o	+	o	+	+	++	o	Acute inflammatory rheumatism during Spanish-American war; "impingement" atherosclerosis of plaque, aorta
20064	W	M	81	o	400	+++	±	o	+	+	+	+	+++	o	Heart attack at 66 yrs.; commissures fused; old adhesive pericarditis
S–3375	W	M	81	+	575	+++	±	+	+	±	++	+	++	o	Inflammatory rheumatism at 14 yrs.; commissures fused; pericarditis, acute, fibrinous

TABLE II

Histological Changes of Rheumatic Character (Monovalvular Cases)

Case number	Age	Heart weight	Aortic Valve				Vascular				Perivascular			Interstitial				Remarks
			Calcification	Arteriosclerosis	Inflammation		"Arteritis rheumatica"	"Fibrinoid" necrosis	Hyperplasia		Aschoff bodies	"Fibrinoid" swelling	Cellular infiltration	Edema	Large mononuclear cells	Scarring	Endocardial thickening	
					Vascular	Cellular			Endothelium	Muscle								
	yrs.	*gm.*																
H.M.H.*	44	700	+++	+	++	+	++	++	+	+++	+	+++	++	++	++	++	+++	Rheumatic fever at 6 yrs.
17455	20	550	+++	+	+++	+++	++	++	+	++	+	++	+	++	++	+++	++	No coronary sclerosis; bicuspid aortic valve
39	40	634	+++	+	+	+	++	++	+	+	o	++	++	+	++	+++	++	Bicuspid aortic valve
S-2363	50	650	++++	+	+++	+	+++	++	++	++	o	++	++	++	++	++	++	Marked ossification aortic valve
19037	53	850	++	+	+++	o	++	+	+	+	+	+	++	+	++	+++	+++	Coronary sclerosis, slight
689	58	500	++	++	++	+	++	++	+	+	+	+	++	+	+++	++	+++	Terminal acute myocarditis; marked coronary sclerosis
14868	60	630	++	o	+	o	+	+	o	+	+	++	+	+	+	+	++	Calcification and inflammation root of mitral leaflet (aortic); positive history (30 yrs.)
691	60	560	+++	+	+++	+	+	++	+	+	o	++	+	++	++	+++	+++	Coronary sclerosis, slight; history measles twice (rheumatic fever?)
S-1137	61	775	+++	+	+++	+	+++	+	++	+	o	+	+	+	++	++	+++	Partial bicuspid aortic valve; history malaria with chills and fever (3 wks.) 1921

* Control case, a known rheumatic polyvalvular case (see Table I).

TABLE II — *Continued*

1410	62	410	+++	+	++	+	+	+	+	++	+	+	++	+	++	++++	++	Tonsillitis and swelling of joints (France 1917–18); marked coronary sclerosis
2450	65	450	+++	+++	++	+++	++	++	++	+	o	+	++	+	++	++	++	Positive history rheumatism; bicuspid aortic valve?
S–1474	66	585	++++	++	++	++	++	++	++	o	+	+	++	+	++	++	+	Chronic pericarditis near origin pulmonary artery
3650	72	650	+++	+++	+++	++++	++	+	+	+++	+	+	++	++	+	++	+	History rheumatic fever Spanish-American war; bicuspid aortic valve
21176	66	450	++	+	+	+	++	++	o	+	+	+	++	++	++	++	+	Rheumatic aortitis; miliary abscesses in myocardium; bicuspid aortic valve
19883	72	400	+++	++	+++	+++	+	++	+	+++	o	+	+	+	+	+	+++	Calcified nodules in sinuses of Valsalva
18905	72	525	++	+++	+	o	++	+	+	+	o	+	+	+	+	++	+++	Bicuspid aortic valve; aortic valve of rheumatic type
14390	74	510	+++	+	++	+++	+	++	+	+	o	++	+	+	o	++	++	Coronary sclerosis (+)
4725	79	725	+++	+	+++	+	++	+	+	o	o	+	+	+	+	+	+++	Acute "inflammatory rheumatism" Spanish-American war; mesarteritis root of aorta
20064	81	400	+++	+	+	++	+++	++	++	++	o	++	++	+	+	++	+++	Commissures fused; old adhesive pericarditis
S–3375	81	575	+++	+	+	+	+	++	+	+	+	+	+	+	+	+++	+++	Inflammatory rheumatism 14 yrs.; aortic commissures fused; acute fibrinous pericarditis and focal myocarditis

nodules, "fibrinoid swelling" or scarring, and mononuclear cellular infiltration and scarring. Interstitial changes are next recorded, a final column being reserved for remarks or miscellaneous data.

There are 19 cases in this group ranging in age from 20 to 81 years. The average age is 62.7 years. There was a positive history of rheumatic fever in 8 cases, or 42.1 per cent (Table I). In spite of an average age of 62.7 years, Aschoff bodies were found in 9 hearts, or 47.4 per cent.

Adherent pericarditis of some degree was present in 6 hearts, or 31.5 per cent, while in 3 cases (15.8 per cent) "soldier spots" were present in the epicardium (Table I).

Arteriosclerosis of the aorta was not a common finding in this group in spite of the advanced age of many of the patients. About one-third of the group showed moderate or marked arteriosclerosis of the aorta. Three patients, or 15.8 per cent, fell into each of the above groups. One patient had no degenerative changes that were observable, while 12, or 63.2 per cent, had only slight changes.

The first case, H. M. H., listed in Table II, is a known rheumatic used as a control, and is not included in the statistical data. In this case there was a history of rheumatic fever at 6 years of age and a moderate (++) lesion of the mitral valve in addition to the marked aortic stenosis. The same physician attended the patient at birth, during his rheumatic infection at 6 years, and again in his final illness. Aschoff bodies are present in the myocardium (Fig. 15). Although only slight cellular inflammation was found near the root of the aortic cusp, nearly all the other criteria of rheumatic involvement as employed in Table II are well represented. Arteriosclerosis of the aorta is slight.

Reference to Table II will show how closely Cases 17455, S–2363, 19037, 689, 691, S–1137, 1410, 2450, S–1474 and 3650 correspond to the control case, H. M. H., as regards their various rheumatic stigmas. Most of these cases show considerable chronic inflammation at the roots of the aortic cusps. In some the changes are largely vascular, while in Cases 17455, 2450, S–1474 and 3650 there is moderate to heavy mononuclear cell infiltration as well. Since only a few sections are taken from a single narrow block through the aortic valve it is likely that cellular infiltration may well be missed in some of these. In 6 of the 10 cases Aschoff

bodies were discovered. Only 2 of the group, Cases 2450 and 3650, had severe arteriosclerosis of the aorta, and both of these present a marked chronic inflammatory reaction at the roots of the cusps. Furthermore, both of these patients had a positive history of early rheumatic infection. It would appear that abundant evidence is at hand favoring a rheumatic origin of the aortic lesion in the 10 cases enumerated.

Case 39: A white male, aged 40 years. Arteriosclerosis was slight. The heart weighed 634 gm. Only slight inflammation was found at the root of the aortic cusp. There was no history of rheumatism. A small collection of mononuclear cells was seen in the myocardium. Other rheumatic stigmas are moderately well represented. There was also a bicuspid aortic valve, a lesion of probable rheumatic origin.

Case 14868: A white female, 60 years of age. In this case the aorta was smooth and there was no inflammation of significance at the root of the aorta. A positive history of rheumatic infection at 30 years of age and the presence of Aschoff nodules in the myocardium place this in the rheumatic group.

Case 4725: A white male of 79 years. There was only slight arteriosclerosis of the aorta, marked vascular and slight cellular changes at the root of the aortic cusp. There was a definite history of acute "inflammatory rheumatism" during the Spanish-American war. A small focus of probable rheumatic mesaortitis (Fig. 17) was found in the root of the aorta. Although the various rheumatic stigmas in the table are poorly represented, there appear to be sufficient data to include this case in the rheumatic group.

Case 20064: A white male of 81 years. The heart was small (400 gm.) and the degree of stenosis moderate. There was slight arteriosclerosis of the aorta and moderate inflammation at the root of the aortic cusps. There was no history of rheumatic infection. Although no Aschoff bodies were discovered other stigmas of rheumatic infection are well represented. There was fusion of the aortic commissures and an old adhesive pericarditis.

Case S–3375: Another white male of 81 years. The heart weighed 575 gm. There was only slight arteriosclerosis of the aorta and slight inflammation at the root of the valve. A history of "inflammatory rheumatism" at the age of 14 years was re-

corded and an Aschoff body was present. The commissures of the aortic valve were partially fused. An acute fibrinous pericarditis and focal myocarditis were present.

There remain in this group 4 cases which grossly at least favored the Mönckeberg type of aortic sclerosis. All had nodular calcified masses within the sinuses of Valsalva. Analysis of the data on these cases, however, leaves little doubt as to their rheumatic origin.

Case 21176: A white male of 66 years. The heart weight was 450 gm., with only slight stenosis. Arteriosclerosis was mild. No record was made of any rheumatic infection. There is only slight evidence of inflammation at the root of the aortic valve. Positive findings of importance consist of an Aschoff body in the myocardium (Fig. 7), a bicuspid aortic valve, subacute myocarditis and thickening along the closing edge of the aortic cusps.

Case 19883: A Mexican male of 72 years. The heart weighed 400 gm. No record was made of any previous rheumatic infection. There was moderate arteriosclerosis of the aorta. Although no Aschoff bodies were found, there is marked chronic inflammation at the root of the aortic valve (Fig. 8) and giant medial hypertrophy of some of the arterioles (Fig. 13). This type of lesion was found in Case H. M. H., and is highly suggestive of rheumatic involvement. Other rheumatic stigmas are somewhat poorly represented.

Case 18905: A white male of 72 years. The heart weighed 525 gm. There is a marked arteriosclerosis of the aorta in spite of a fairly severe grade of stenosis; little or no inflammation was found at the root of the aortic cusps. The aortic cusps, however, are broadly thickened at the closing edges and much narrower at their bases, except where calcified nodules are present. Thickening along the closing edges is usually regarded as the result of rheumatic infection. Furthermore, there is a bicuspid valve which is strongly suggestive of rheumatic disease.

Case 14390: A white female of 74 years. The heart weighed 510 gm. There was a history of rheumatism of the left leg 20 years ago, which we consider of little significance. A mild arteriosclerosis of the aorta was present but with a fairly well marked inflammation of the aortic root. No Aschoff body was found. The various other rheumatic stigmas are but fairly well represented,

partly we believe because the heart muscle is poorly preserved. The absence of interstitial mononuclear cells may perhaps be accounted for in this way. This is our weakest case as regards positive rheumatic findings. The presence of only a mild arteriosclerosis of the aorta and coronary arteries militates against including it in the degenerative group of Mönckeberg.

Discussion

An analysis of the data in the group of 31 cases of nodular calcified aortic valve presented in this study indicates a rheumatic origin in practically all. In the one or two cases that seem a bit doubtful there is more evidence favoring a rheumatic etiology than there is for the Mönckeberg type of sclerosis. It is possible that some of the patients in whom a bicuspid aortic valve was found had congenital rather than acquired aortic lesions. If such cases exist the rheumatic involvement would be secondary to the congenital lesion.

A brief review of the important factors favoring a rheumatic etiology in the group may be relevant.

Thirty-nine per cent of the whole group had chronic fibrous or calcific changes in two or more valves. The valve most frequently involved in addition to the aortic was the mitral, which showed changes commonly associated with rheumatic disease of the heart. This figure is only slightly less than in the series studied by Clawson, Noble and Lufkin,[1] who found 44.5 per cent with polyvalvular lesions. There was a positive history of previous rheumatic infection in 15 of our cases, or 48 per cent. In 9 patients no note was made concerning such an episode. If we assume that about 50 per cent of the 9 patients also had positive histories the total would then be 19.5, or 63 per cent.

In sixteen hearts (51.6 per cent) a chronic inflammation of at least moderate degree was found at the root of the aortic cusp. Of the polyvalvular cases 5 out of 9 cases examined, or 55.5 per cent, showed such inflammation, while 11 of the 19 monovalvular cases had such changes, making 58 per cent for the latter group.

There are 6 cases belonging to the monovalvular group which show only slight evidence of inflammation at the root of the aortic cusp. It must be remembered, however, that the sections examined cover no more than perhaps 1/600th of the entire aortic

ring. Marked inflammation may be present and yet not show in the few sections examined. Examining these 6 cases further we find in 2 of them (Cases 14868 and S–3375) a history of rheumatic fever and the presence of Aschoff nodules; another case (21176), in addition to a myocardial Aschoff nodule, revealed a rheumatic nodule in the aortic root and a bicuspid aortic valve; 2 additional cases had bicuspid valves, 1 of these (Case 18905) showing marked arteriosclerosis of the aorta. Sections of the aortic valve show a rheumatic type of lesion in the cusp, *i.e.* marked thickening along the closing edge. The other case which had a bicuspid valve (Case 39) exhibits a fairly good array of rheumatic stigmas. The final case (20064) had an old adhesive pericarditis, the aortic commissures showed some fusion and the rheumatic stigmas were fairly well represented. Rheumatic arteritis was conspicuous among these.

Since we have considered the factors favoring a rheumatic origin, what are some of the stigmas relating the process to Mönckeberg's aortic sclerosis? Atherosclerosis of the aorta is quite essential since this process is supposed to extend from the aorta to the cusps through their attachments. Only 3 cases of the monovalvular group (2450, 3650 and 18905) were found to have a marked atheromatous involvement of the aorta. Of these, Case 2450 had marked chronic inflammation at the root of the aortic cusp; there was a positive history of rheumatic fever; there was a bicuspid aortic valve, and a goodly array of rheumatic stigmas. The heart was small, 450 gm., and the degree of stenosis was slight. Where stenosis is mild there is no deterrent to the development of atheromatous changes so far as wear and tear are concerned. On the other hand, a small heart indicates that the blood pressure was not appreciably increased.

Case 3650 showed marked inflammation at the root of the cusp, there was a positive history of rheumatic infection, Aschoff bodies were present, and there was a bicuspid aortic valve and abundant rheumatic stigmas as shown in Table II. Case 18905 was not so definitely rheumatic as the other 2 but it had a bicuspid aortic valve and the aortic cusps were thickened along their closing edges. Thus we see that in the 3 cases showing the most atherosclerosis of the aorta and which might, therefore, belong in the group described by Mönckeberg, 2 of the cases are conspicuously rheumatic in origin. The 3rd case not only does not conform to the

criteria outlined by Mönckeberg, but exhibits several characteristics of chronic rheumatic infection.

The microscopic findings in the hearts of our older patients indicate an activity scarcely to be expected. We, therefore, wish to emphasize the great chronicity or "in-dwelling" character of the rheumatic infection.

Summary

1. Thirty-one unselected cases of nodular calcific aortic stenosis have been studied from the standpoint of etiology. In 12 cases, or 38.7 per cent, there was a healed lesion of the mitral valve in addition to the aortic stenosis. In 19 cases, or 61.3 per cent, there was a solitary lesion of the aortic valve.

2. There was a positive history of a previous attack of rheumatic infection in 15 cases (48.4 per cent).

3. Nineteen hearts having a single aortic lesion were sectioned according to the standard method described by Gross, Antopol and Sacks.[8] Histological studies revealed substantial evidence of rheumatic involvement in every case.

4. The average age of the patients with a combined mitral and aortic lesion was 59.3 years. The group with a single aortic lesion averaged 62.7 years. The average cardiac weight in the former group was 618 gm., while in the latter it averaged 571 gm. Males outnumbered females in the first group 3 to 1, and in the second group 4 to 1.

5. In 8 patients of the monovalvular group (42.1 per cent) a bicuspid aortic valve was found. Strangely none was found in the mixed group.

Conclusions

We must conclude from this study that the cardiac changes in the cases here presented all appear to have a common etiology. If our interpretation is correct the changes described conform to the criteria generally accepted for chronic rheumatic infections of the heart.

Note: We acknowledge our indebtedness to several colleagues who furnished material from their respective hospitals. We are especially grateful to Dr. A. G. Foord who supplied us three specimens together with the clinical data.

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DESCRIPTION OF PLATES

PLATE 130

FIG. 1. Case S–2950. Gross photograph of heart showing the thickened calcified cusps of the aortic valve with fusion of the commissures. The aorta is relatively smooth. The mitral valve is moderately involved.

White male, 56 years of age, with a history of rheumatic fever in 1907. Heart weight 580 gm. × 0.5.

FIG. 2. Case 46. View of the aortic valve from above with the aorta removed. Calcified nodular aortic stenosis is present with only a slit-like lumen remaining.

White male, 60 years of age, with a history of rheumatic infection at 12 years. Heart weight 715 gm. × 0.75.

FIG. 3. Case S–2363. A nodular calcified aortic valve with a slit-like opening similar to that shown in Figure 2. The aorta is smooth except for a slightly raised linear atheromatous "impingement" plaque which is directly in line with the narrow opening in the valve.

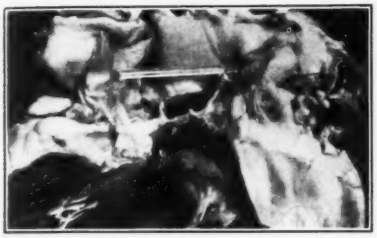
White male, 50 years of age, with a history of rheumatic infection at 6 years. Heart weight 650 gm. × 0.75.

FIG. 4. Case S–1474. Thickened, nodular calcified aortic cusps showing an incomplete division between the right and the posterior cusps (bicuspid valve). The aorta is smooth.

White male, age 66 years, with no definite history of rheumatic infection. Heart weight 585 gm. × 1.

FIG. 5. Case 3650. An Aschoff body consisting of small mesenchymal cells. One or two larger cells are present.

White male, age 72 years, with a history of an acute rheumatic infection during the Spanish-American war. Heart weight 650 gm. × 200.



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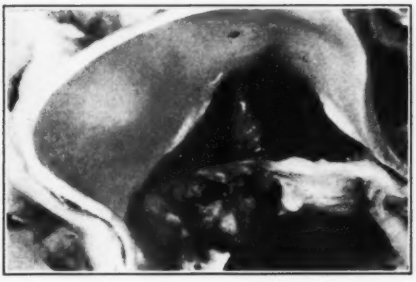

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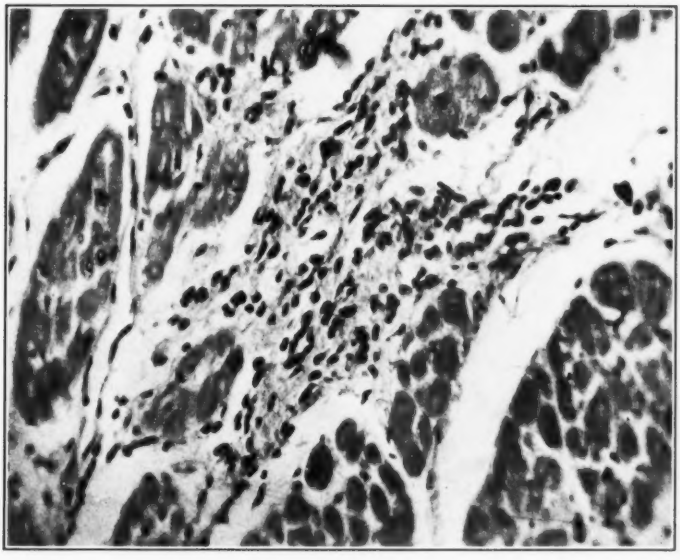

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Plate 131

Fig. 6. Case 3650. A fibrillar, polarized Aschoff body composed of fibroblasts and larger mesenchymal cells. Same patient as in Figure 5. × 200.

Fig. 7. Case 21176. Perivascular collection of mesenchymal and large mononuclear cells with a few polymorphonuclear leukocytes present. There is hemorrhage in the lower part of the field. A subacute myocarditis was present in this case.

White male, age 66 years, with no record of rheumatic infection. Bicuspid aortic valve. Heart weight 450 gm. × 200.

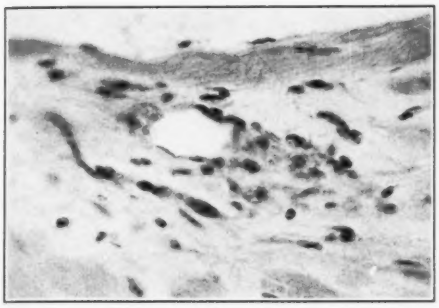
Fig. 8. Case 19883. Chronic inflammation near the root of the aortic valve. Many large dilated capillaries and many large mesenchymal cells are seen.

White (Mexican) male, age 72 years, with no history of rheumatic infection. Heart weight 400 gm. × 100.

Fig. 9. Case 3650. Ossification of calcified material at the root of the aortic valve. Note the marrow spaces. Dense round cell infiltration is seen in the lower part of the field. Same patient as in Figures 5 and 6. × 100.

Fig. 10. Case 21224. Myxomatous type of connective tissue proliferation in one of the valve leaflets. Many large plump nuclei are present similar to the picture frequently seen in the more acute rheumatic infections.

White male, age 86 years. The mitral valve shows moderate involvement. Heart weight 560 gm. × 100.



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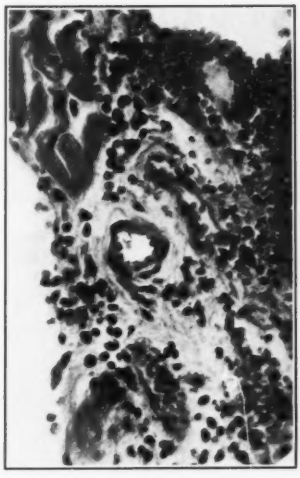

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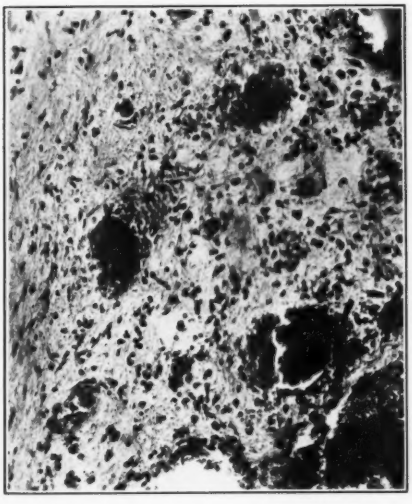

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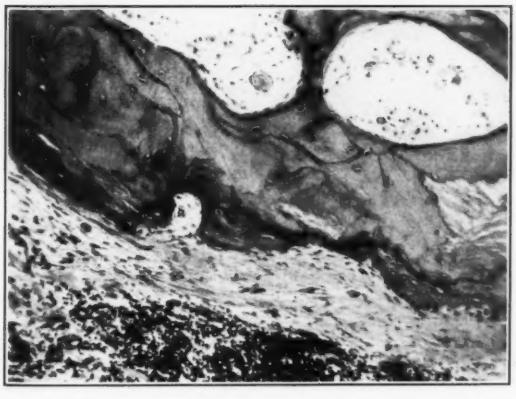

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PLATE 132

FIG. 11. Case S–2363. Necrosis of arterial wall with "fibrinoid" swelling in the perivascular space. Some of this material stains with the Weigert fibrin stain. Same patient as in Figure 3. × 100.

FIG. 12. Case 17455. Disorganization of a small coronary artery commonly seen in rheumatic infections (arteritis rheumatica).

White female, age 20 years. An Aschoff body and a bicuspid aortic valve is present but there is no history of rheumatic infection. Heart weight 550 gm. × 150.

FIG. 13. Case 19883. Giant medial hypertrophy of one of the small coronary arteries. Same patient as in Figure 8. × 100.

FIG. 14. Case 4725. Edema of media in coronary arteriole ("état reticulaire").

White male, age 79 years, with a history of acute rheumatic infection during the Spanish-American war. Heart weight 725 gm. × 150.

FIG. 15. Case H. M. H. Aschoff body in the myocardium.

White male, age 44 years, with a history of rheumatic fever at 6 years of age. Heart weight 700 gm. × 100.

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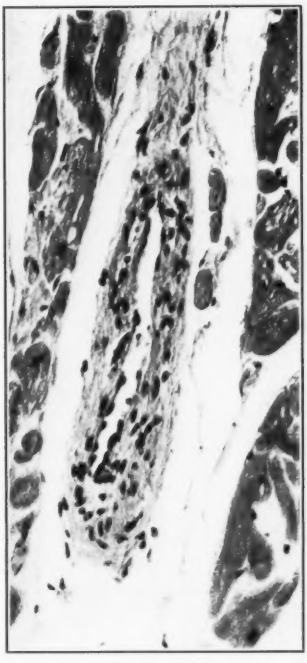

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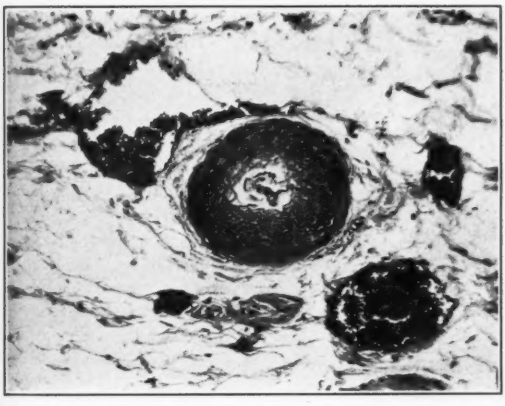

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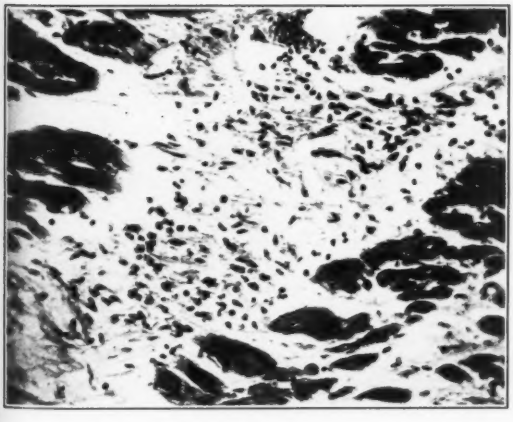

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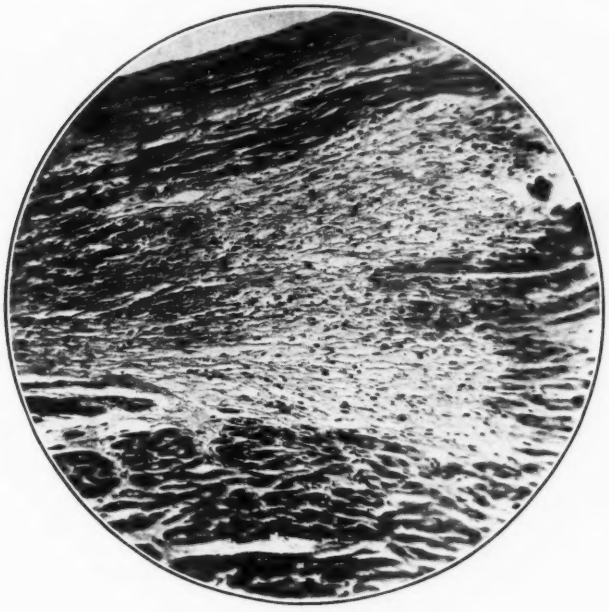
Plate 133

Fig. 16. Case 17455. Scar in the myocardium (posterior papillary muscle). Numerous small scars of this type are due to rheumatic involvement of the small arteries (see Figure 12). The main coronary arteries are normal. Same patient as in Figure 12. × 100.

Fig. 17. Case 4725. Small inflammatory area in the root of the aorta (mesaortitis). Same patient as in Figure 14. × 150.



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17

STUDIES ON THE INVOLUTION OF THE FETAL CORTEX OF THE ADRENAL GLANDS *

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Since Thomas' [1] recognition in 1911 that during the 1st year of life there is regularly a complete degeneration of the inner zone of the adrenal cortex, there have been a few papers published dealing with the histology of the process, but there has been no tabulation of the range of "normal" variation and little attempt at correlation of unusual alterations in the involuting gland with pathological changes in other organs. The present review of cases is given to show the range of variation found in the adrenal glands in 217 routine autopsies on infants and children, and to contribute to the knowledge of possible forces acting to modify the rate of involution of these glands.

The adrenals at birth are relatively large structures, about one-third the size of the kidneys, and are made up of a fairly firm, yellowish gray cortex and a wide, brownish red inner portion. The gland is composed of an outer or true cortical part consisting of a narrow zone of small deeply staining cells arranged in ovoid or acinar groups, and an inner portion made up of large polyhedral cells which are frequently fat-containing, take a pale acidophilic stain and are in no definite arrangement. This inner part is the fetal cortex which is destined to disappear after birth. In the most central region a few deeply staining medullary cells may be seen. The primary inner zone, or fetal cortex, is present as a definite structure by the 4th fetal month [2] and continues with no morphological change up to the time of birth. Hyperemia is a feature of the fetal cortex at all times and is maximal at the time of birth.[3]

The involution of the inner or fetal zone of the adrenal cortex has been studied by Lewis and Pappenheimer [4] in 100 cases. A summary of their observations is as follows:

(*a*) In the first few days of life the innermost cortical layer becomes increasingly hyperemic and the great filling of the capil-

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laries loosens the columnar alignment of the cells and produces displacements, compressions and degeneration of individual cells; massive degeneration and sequestration of the fetal cortex become manifest in the 2nd week of life. By the end of that week degeneration is well advanced.

(*b*) Completely established involution is seen at the end of the 1st month and lasts throughout most of the 1st year of life. The degenerating zone becomes sharply marked off from the superficial cortex and from the medulla by what appears to be a layer of connective tissue. After the 5th or 6th week this degenerating zone of cells rapidly decreases and the fibrous zone becomes clearly defined and shows shrinkage.

(*c*) The third stage is reached when the resorbed parenchyma is finally replaced by connective tissue which surrounds the medulla like a capsule. This zone of what appears to be connective tissue may be merely the stroma of the fetal cortex which becomes prominent as the parenchymatous cells disappear.

(*d*) The fourth stage is reached by the end of the 1st year and continues to adult life. It is manifested by the gradual disappearance of the narrow strip of fibrous tissue between the cortex and the medulla. There is no growth or regeneration of true cortex, according to Lewis and Pappenheimer,[4] during the first 3 years of life and the adrenal gland attains morphological stability in the 8th year.[5]

The present work is based on a study of the adrenals from 44 stillborn infants, 64 living infants less than 2 days of age, 33 infants who died between the ages of 2 days and 1 month, 32 infants who died between the end of the 1st month and 1 year, and 44 children who lived from 1 to 13 years. A total of 217 cases was included, macerated fetuses and infants and children on whom the autopsies were incomplete having been omitted. Routine hematoxylin and eosin stained paraffin sections have been used in all cases; special stains have been tried in some instances but have not contributed any significant additional information. The stillborn infants and those who died before 2 days of age have been classified according to maturity, using total length as the index, and all who lived more than 2 days, as well as all older children, were classified by age. No attempt was made to select cases in

respect to cause of death, but all pathological processes seen at autopsy have been considered in making the analysis.

Group I consists of 44 stillborn infants and fetuses. Group II consists of 64 infants who were born alive and were less than 2 days of age at the time of death. Since careful study of all the material from both groups showed a close parallelism of all findings, indicating that there was no appreciable progression of the involution of the fetal cortex in the first 2 days of life, Groups I and II (108 cases) will be considered together.

TABLE I

Weight Distribution of Adrenal Glands from a Series of 108 Cases

Length of fetus	Average weight of adrenals (both glands)	Limits of variation in weight of glands
cm.	*gm.*	*gm.*
25–30	3.8	2.5–6.0 (4 cases)
31–35	3.3	1.5–5.0 (10 cases)
36–40	4.0	1.5–5.7 (15 cases)
41–45	5.7	2.2–11.0 (27 cases) larger glands in newborns
46–50	6.4	2.8–11.0 (29 cases) larger glands in newborns
51–55	10.3	4.0–15.0 (17 cases) larger glands in stillborns

Six cases were omitted because of extreme deviation from the remainder of the group.

The weight of each adrenal gland in a full term infant has been given as 4.7 to 5 gm.[5] Table I shows the weight distribution in the present series of 108 cases.

From the data in Table I it appears that the rate of adrenal growth increases rapidly as the fetal length nears 50 cm., or term, and the individual variation increases after the fetus passes about 45 cm. in length. Scammon [6] graphed the average adrenal weights in 1087 pairs of glands from fetuses and plotted the adrenal weight against total body weight. His curve is similar to the one constructed from the data in the present study.

Analysis of the present series of cases shows no evidence of any sex variation in either the size or appearance of the adrenal glands. Morphologically the glands all show a narrow outer band of cells, appearing in groups or acini near the capsule and taking on a more columnar arrangement as the central portion is approached. These cells contain distinct, round or oval, deeply staining nuclei

and a moderate amount of granular neutrophilic cytoplasm. The cells of the outer zone gradually give way to slightly larger polygonal acidophilic cells with less prominent nuclei which are arranged in columns. The latter cells make up the fetal cortex. There is considerable variation in the distinctness of the boundary between true and fetal cortices; a few glands show a clear line of demarcation but the majority show an indistinct transition. The fetal cortex is two to five times as wide as the true cortex and in its central part is usually congested with blood. In 6 cases the cells of the fetal cortex show no evidence of involution whatever, but in the majority degenerative changes are seen in the cells located in the midportion of the gland. This degeneration is manifested by vacuolization, acidophilic staining, loss of nuclear differentiation and, finally, by dropping out of the cells. A few medullary cells are usually present either singly or in small groups in the center of the gland.

In the outer zone of the true cortex the acinar arrangement of the cells is frequently emphasized by the presence of lacunae containing an amorphous precipitate. These lacunae are seen almost invariably up to about the 8th month of fetal development, or until the fetuses measure 40–45 cm. in length, and may also be found occasionally in more mature infants, sometimes persisting for several months of extrauterine life. It is probable, therefore, that the presence of such lacunae in older individuals is a persistence of the fetal condition and pathological only as an indication of delayed development.

Since slightly more than 89 per cent of the adrenals studied presented similar changes microscopically, namely, variation from entire absence of degeneration to a minimal destruction of cells in the most central part of the fetal cortex, I have chosen to consider these to be "normal" glands. The remaining 11 per cent show unusual degrees or types of involutionary change in the fetal cortices and have been placed in a "special" group for more careful consideration as to whether they should be designated as truly abnormal or merely variations of the "normal."

In the "special" group of 12 cases 7 show only a moderate deviation from the "normal." One of these cases was included because of the small size of the adrenals but showed no microscopic abnormality, and the remaining cases showed a somewhat

wider zone of degeneration than usual with numerous empty cell spaces centrally located. Of the 5 remaining cases, in 4 there was degeneration involving one-third or more of the fetal cortex, and in 1 there was almost complete involution comparable to that usually seen at about 1 month of age. In these 12 infants the cause of death varied from birth injury to sepsis and there was no apparent association between the condition of the adrenal glands and any other pathological findings, except possibly in the one case showing the greatest deviation from "normal." In this case there were bilateral bean-sized cysts lying in the choroid plexuses of the lateral ventricles of the brain and containing firm yellow homogeneous material. The frequently observed relationship of anencephaly and involution of the adrenals is undoubtedly significant,[5, 7, 8] and the fact that the one case in this series showing extreme involution presented an abnormality of the choroid plexus suggests some relationship between adrenal involution and pathological changes in the central nervous system.

Because the majority of cases show only minimal degeneration of the fetal cortex at or immediately following birth, I have not considered as abnormal the 6 cases which showed an absence of such involutionary changes, although it is possible that such a delay is of importance. Lewis and Pappenheimer [4] reported 2 syphilitic infants in whom the involution of the fetal cortex appeared to be delayed, but I have not been able to discover any evidence of syphilis in this series. The fact that even a small percentage of cases shows a definite increase in the amount of degeneration of the cells of the fetal cortex in both stillborn and liveborn infants suggests that involution may be initiated before birth and cannot be invariably associated with the act of birth itself. Also, this is borne out by the fact that anencephalic monsters, though they show extreme adrenal involution, are usually born at or near term.

In addition to the variations which have been mentioned, I have observed one condition demonstrating an apparently significant correlation. In 8 cases in which there was a mild degree of hyperkeratosis of the skin the adrenal glands were unusually large; in 3 cases the glands were above average in size though not beyond the range of "normal" variation, but in 5 cases the glands were outside even the range of variation for all other cases. It has been observed [7] that in exfoliative dermatitis of the newborn there

may be adrenal hypoplasia, and the fact that two diseases of the skin show adrenal abnormalities suggests that there may be a relationship between these glands and skin metabolism.

Group III consists of 33 infants who lived at least 3 days and not more than 1 month. In this period massive degeneration and sequestration of the fetal cortex of the adrenals is initiated and reaches its greatest extent. The cases are classified according to age rather than maturity. Because of the relatively small number of infants in this group it is not possible to do more than describe the progress of involution as seen in representative cases from similar age groups.

In the previous groups (I and II) it was shown that adrenal weights increased markedly as the fetal length approached 50 cm.

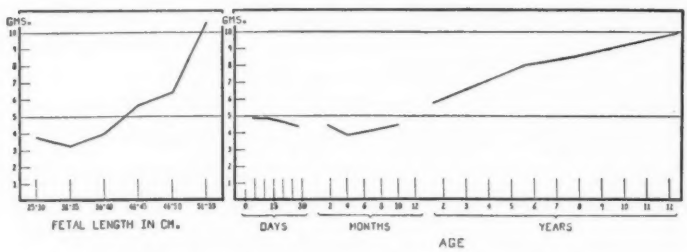

CHART 1. Showing the combined weights of the adrenal glands in relation to fetal length in newborn infants and in relation to age in older children.

In the present group maturity has not been used as an index and the infant lengths vary considerably. The majority lie between 40 and 50 cm. and it is estimated that the average adrenal weight on the 2nd day of life lies between 4 and 10.3 gm. From the 3rd day onward the average weight of the glands approximates 4 or 5 gm. In fact, there is a remarkable constancy to this figure for the average weight of the adrenals throughout the rest of the 1st year of life. Scammon [6] has presented similar figures for this age period. Individual variation is greatest in younger infants, lessens gradually, and by 1 month is about half as great as on the 3rd day (Chart 1).

Microscopic study of the material reveals no indication of change in the structure of the glands between the 2nd and 3rd days, and on the 3rd day of life the fetal cortex is entirely within

the limits of what has been described for newborn infants. By the 4th day degeneration has spread outward to include about one-third, and by the beginning of the 2nd week to include about four-fifths of the fetal cortex. By this time, the beginning of the 2nd week, many cells have disappeared from the central part of the gland and the stroma has become more prominent, sometimes appearing as fibrous tissue arranged in parallel strands. In the 2nd and 3rd weeks the degenerative changes involve the entire fetal cortex which is transformed into a rather disorganized appearing mass of tissue made up of strands of stroma, scattered degenerating cells and bits of débris. The degree of hyperemia is variable. By the 3rd week there is widening of the true cortex up to about twice the thickness seen in newborn infants, as well as proliferation of the medullary cells. The true cortex stains more deeply and the involuting tissue more faintly, so that the boundary between the two becomes increasingly definite. By the end of 1 month the medulla is prominent, the true cortex has increased two or three times in width, and the fetal cortex is represented by scattered degenerating cells lying in an irregular stroma. The true cortex and the medulla both increase as the fetal tissue disappears. Thus, the statement of Lewis and Pappenheimer [4] that there is no growth of the true cortex during the first 3 years of life is not substantiated by my findings.

Of the 33 cases studied in Group III, 28 show the above changes. In the 5 remaining cases the following points were noted. One infant (4 days of age) showed markedly advanced adrenal involution; the only suggestive associated finding was an unusual prominence of the interstitial cells of the testis. Four cases showed a somewhat delayed involution and 1 case in particular (age 1 month) showed only about half of the fetal cortex destroyed. In 2 additional cases there was kernicterus of the brain coincident with adrenal modification. In these the fetal cortices of the adrenals contained groups of hyperchromatic cells which were arranged radially through the inner half of this region. Degenerating cells in the adrenals were pigmented in a manner apparently similar to the pigmented brain cells.

Whereas in Groups I and II the majority of infants suffered from birth trauma and the minority from infection, in Group III most of the infants died as the result of severe infectious disease.

It has been stated by many investigators that in the presence of infection there may be an increase in the size of the adrenal glands and, if so, it may be that the weights given in this series are greater than normal. It has not been possible to correlate adrenal involution with the cause of death, and there is no evidence that either sex or prematurity are associated with any modification of the involutionary process.

Group IV includes 32 infants from 1 month to 1 year of age. During this period there is continuation of the degeneration and sequestration of the fetal cortex.

As shown above, the combined weights of both adrenals approximated 4 or 5 gm. for infants from 3 days to 1 month of age. In the present group there is no change in the average weights and no appreciable difference in the degree of variation observed. Microscopic study of the material reveals a gradual decrease in the number of remaining cells of the fetal cortex, and a fairly rapid decrease in the amount of stroma up to about 2 months of age; after that time there is a slow decrease up to the 5th or 6th month until only a narrow rim of stroma is present which remains quite constant up to about 1 year of age. Variations in the width of the connective tissue layer appear to be due to hyperemia or edema, particularly after the 3rd month. During the entire period covered by this group of cases there is a steady increase in the size of the medulla and of the true cortex, the latter being manifested by increase in both width and lateral growth which tends to flatten and spread the glands.

There is no evidence of any sex differences or of any correlation with other pathological findings. All of the infants died of infectious disease.

Of the 32 cases presented, 29 show the changes already described and 3 show delayed involution. One of these 3 cases (age 1 month) retained a wide zone of fetal cortex in which there were slight degenerative changes. Two cases (age 3 months) also showed a considerable amount of fetal cortex at a period when the majority showed practically complete involution.

Group V consists of 44 children from 1 to 13 years of age. As noted above, Lewis and Pappenheimer [4] believed that the adrenals show no growth of the true cortex until the 3rd year, and Goldzieher [5] stated that they attain morphological stability at about

the 8th year. Study of this series of cases does not warrant such conclusions, as growth of the true cortex is microscopically evident throughout the entire life of the child. However, total gland weight begins to increase after the end of the 1st year, is fairly rapid through the 5th year, and diminishes in rate after that time. In the first 5 years the combined weight of the adrenal glands almost doubles, whereas in the next 5 years it increases only one-fifth as rapidly.

TABLE II

Average Weights of the Adrenal Glands Correlated with Age

Age	Average weight of adrenal
yrs.	*gm.*
1	4.7
2	5.8
3½	6.9
5	8.0
11	9.3

Microscopically there is a progressive increase in both medullary and true cortical tissue throughout the entire period from birth to 11 years. The connective tissue stroma decreases as this growth occurs, and by 2 years this stroma appears as a thin line, by 3 years it has disappeared in occasional cases, and by 5 years no glands show any appreciable amount.

DISCUSSION

Analysis of all the cases in the present series indicates that at birth the adrenals are made up of a narrow zone of true cortex, a wide zone of fetal cortex, and a few medullary cells. The fetal cortex begins to degenerate at, or just previous to, birth. The degeneration proceeds rapidly for 2 or 3 weeks with a loss of cells and increasing prominence of the stroma which takes on the appearance of a zone of connective tissue. Growth of both medullary and true cortical tissue proceeds fairly constantly in fetal and postnatal life and, after the involution of the fetal zone, its discarded stroma is slowly reutilized as a framework for the newly forming tissue. There is no evidence that growth of either true cortex or medulla is sporadic, and good evidence that it is a continuous process equalizing the destruction of fetal cortex for a

time and then becoming manifest in the increasing size of the glands.

A few cases showed accelerated involution and about the same number showed delay; it may be that these were merely variations of "normal," but it seems more probable that they reflected the action of some undetermined modifying factor. The range of variation seen was greater than would be expected from the literature and was most marked between the 1st week and the 1st year of life. The glands of newborn and stillborn infants showed similarity, but there was enough variation to indicate that involutionary changes may begin before birth or may be delayed for some time.

Goldzieher [5] stated that involution was due to altered physiology at birth and was associated with changes in oxygen tension. The fact that even a few cases showed a marked involution before birth, namely anencephalics and a few other cases in which there was no associated brain disorder, tends to disprove this theory. Bonar [9] suggested that there was a relationship between the adrenal changes and heat production or regulation of respiration, but again the anencephalics and others which showed early involution of the fetal cortex tend to disprove this statement. Grollman,[7] as well as Broster,[10] stated that the fetal cortex is composed of androgenic tissue and is concerned with the sexual development of the infant. These writers have presented considerable evidence for their beliefs, namely that definitely androgenic tumors of the adrenals show specific granules which are similar to granules seen normally during a short period of fetal life, that fetal cortical tissue appears to persist in hermaphrodites,[7, 11] that pituitary gland abnormalities modify the size and structure of the adrenals, and that the adrenal is modified by castration as well as by injections of sex hormones. In the present study I have found no evidence of any sex differences in the adrenal glands and, therefore, feel that further chemical and physiological analysis of the tissue must be done before androgenic activity can be assigned to the fetal cortex. It is also possible that the fetal cortex contains some "x" substance necessary to protect the fetus from maternal hormones or other substances which are withdrawn at birth. This latter suggestion is very indefinite but seems to me to be deserving of further chemical and biological investigation as the fetus appears to be little affected by the hormonal modifications regularly found

in the mother during pregnancy. There is evidence, also, that there is a possible relationship between the adrenal glands and metabolism of the skin, and there should be more investigation of this relationship.

Summary

1. Adrenal glands have been studied in a series of 217 fetuses, infants and children. Particular attention has been paid to the involution of the fetal cortex.

2. There are marked individual variations in the rate of involution of the fetal cortex.

3. There is steady growth of the true cortex and medulla of the adrenal glands throughout infancy and childhood. This growth first replaces the destroyed fetal cortex and later is manifested by increase in the size of the glands.

4. The function of the fetal cortex is still doubtful and none of the theories so far presented in the literature explain all of the facts observed. Further chemical and biological studies are necessary to shed further light on the question.

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THE RESPONSE OF THE CENTRAL NERVOUS SYSTEM TO THE APPLICATION OF CARCINOGENIC HYDROCARBONS *

II. Methylcholanthrene

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The present report describes a continuation of the attempt to produce intracranial tumors in mice by means of carcinogenic hydrocarbons. In the first experiment [1] cholesterol pellets containing 5 per cent of dibenzanthracene were placed in the brains of 81 stock mice. Only 1 tumor, an extracranial fibrosarcoma, was produced, though 40 mice survived longer than the 282 days required to obtain this tumor.

Subsequent to this report Seligman and Shear [2] described a very successful experiment using methylcholanthrene. These workers implanted pellets of pure fused methylcholanthrene deeply into the brains of 20 C_3H strain mice. Over a period of 511 days 13 tumors were obtained, 11 of which were considered to be gliomas and 2 fibrosarcomas.

These authors also reported the following experiments with negative results. Approximately 1 mg. of methylcholanthrene suspended in normal saline was injected into the cistern of 13 strain D mice. No tumors were obtained after 6 months. Approximately 2 mg. of methylcholanthrene in saline was similarly injected into the cistern of 10 male strain A mice, with no tumors at the end of a year. Eighteen strain D mice received 0.1 mg. of methylcholanthrene in lard into the cistern, with no tumors after 18 months. Five young rats received 1 mg. of methylcholanthrene in saline suspension into the cistern, but no tumors had appeared at the end of 18 months. A pellet of fused methylcholanthrene weighing 5 mg. was implanted into the motor cortex, and a similar one into the axillary space of a young cat. After its death from pneumonia a year later both pellets were found intact with no trace of neoplastic reaction about them.

In addition Seligman and Shear cite the experiment of Bertrand

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and Gruner who injected benzpyrene in oil into the brains of rabbits, with negative results after a year. They also quote the following interesting personal communications. Zylberszac, at the Laboratory of Pathological Anatomy, Faculty of Medicine, University of Brussels, implanted crystalline benzpyrene into the frontal cortex of 35 rats, with no tumors obtained after 14 months. Scherer, at the Institute Bunge, Berchem-Anvers, Belgium, injected a 1 per cent solution of benzpyrene in lard into the frontal lobes of 70 rats. Fifty of these received multiple injections. Twenty-seven rats lived more than 200 days. The experiment was terminated at 300 days with no tumors having been obtained. In fact it appeared that lard alone provoked as vigorous a granulomatous foreign body reaction as did the benzpyrene solution. Andervont of the U. S. Public Health Service injected approximately 0.05 mg. of dibenzanthracene suspended in horse serum intracerebrally in 35 C_3H strain mice. Nine animals lived for more than 13 months, but no trace of tumor was found in any.

Technic

The technic of implantation of the carcinogenic pellets and of preparation of the microscopic sections differed only in minor details from that described in the first report.[1]

The methylcholanthrene employed was obtained from the Eastman Kodak Company. Pellets of 10 per cent concentration were prepared by fusing together 9 parts of chemically pure cholesterol and 1 part of methylcholanthrene, and drawing the molten solution up into oiled capillary tubes of 1 mm. bore. After solidifying, the rod of material was forced out and cut into cylindrical pellets averaging 2 mm. in length. These pellets proved rather more friable and difficult to handle than the 5 per cent dibenzanthracene used previously. Their implantation was greatly facilitated by the use of an injector consisting of a glass pipette and plunger that could be operated with one hand.

The same histological technic as previously described was employed in this experiment. The brain and calvarium were fixed whole in Zenker's fluid containing 10 per cent of acetic acid, thus obviating the necessity of decalcifying the skull. A transverse slice bearing the operative field and pellet was embedded in paraffin, and sections were cut at 5 μ and stained routinely with hematoxylin

and eosin, phosphotungstic acid hematoxylin, Masson's trichrome stain, and Foot's modification of the Hortega impregnation method for reticulum. Admittedly this procedure made it impossible to apply the Spanish impregnation methods for neuroglia. However, the economy of tissue and the better micro-anatomical preparations so obtained more than compensated for the lack of neuroglial impregnations.

Experimental Data

Pellets containing 10 per cent of methylcholanthrene were implanted into the right parietal cortex of 110 stock albino mice. The colony consisted of about equal numbers of the two sexes, and the animals were of unknown age though certainly adult. Six were killed by operative trauma. An additional 5 disappeared sometime during the latent period, leaving a total of 99 actually taking part in the experiment. The first tumor was recognized on the 101st day. Eighty-seven animals survived into this tumor-bearing period. The experiment had to be terminated and all surviving animals killed at the end of 193 days. During this interval tumors of various sorts were produced in 28 of the 87 available mice. Significant data on the tumor-bearing mice are presented in Table I, arranged in chronological order of the appearance of the tumors.

Certain generalizations may be deduced from the tabular data. The sex of a third of the tumor-bearing animals is unknown because their tumors were internal or too small for gross recognition at autopsy. Such tissues were embedded and mounted in groups according to postoperative age without individual identification. The 18 animals of known sex are evenly divided between males and females. Since the colony employed was also composed of an equal number of males and females, it appears that sex exerted no determining effect on the occurrence of tumors. Mesodermal tumors were found in 7 males, 6 females and 4 unknown. Gliomas seemed to be more frequent in females, 6 to 2, but the sex of 7 mice with gliomas is unknown.

Of the total of 32 tumors produced, 17 are considered to be of mesodermal and 15 of neurectodermal origin. As shown in Table I the gliomas seem to accumulate in a group at the end, an expression of their more tardy development. The significance of this

arrangement is more clearly revealed when the tabular data are expressed in graphic form by plotting the number of tumors against the time required for their production (Chart 1).

It is apparent, since both curves show no terminal flattening, that the experiment was ended before the carcinogenic effect of

TABLE I

Number of mouse	Sex	Day post-operative	Tumor	Symptoms
18–3	F	101	Rhabdomyosarcoma	Bulging, comatose
12–1	F	108	Medulloepithelioma	Bulging
15–2	F	113	Fibrosarcoma	Lethargic, circling
16–2	M	114	Leiomyosarcoma	Mass above eye
19–1	M	116	Rhabdomyosarcoma	Mass above eye
2–2	M	117	Fibrosarcoma	Bulging, lethargic
14–1	M	118	Rhabdomyosarcoma	Slightly bulging
5–1	F	127	Medulloblastoma	Bulging, lethargic
4–4	F	130	Fibrosarcoma	Bulging, lethargic
13–2	M	133	Fibrosarcoma	Bulging, convulsion
8–3	F	153	Fibrosarcoma, medulloblastoma	Bulging, lethargic
7–3	M	171	Fibrosarcoma	Lethargic, marantic
17–2	M	180	Medulloblastoma	None, end of experiment
16–4	?	181	Sarcoma, osteogenic ?	" " " "
D–1	?	184	Fibrosarcoma	" " " "
14–3	M	184	Fibrosarcoma	" " " "
C–3	?	186	Fibrosarcoma, glioblastoma multiforme	" " " "
C–6	F	186	Leiomyosarcoma	" " " "
B–2	?	187	Fibrosarcoma	" " " "
B–4	?	187	Glioblastoma multiforme	" " " "
B–7	?	187	Medulloblastoma	" " " "
B–8	?	187	Medulloblastoma	" " " "
8–4	F	187	Fibrosarcoma, medulloblastoma, glioblastoma multiforme	Blind ?, circling
9–3	M	187	Glioblastoma multiforme	None, end of experiment
A–3	?	192	Glioblastoma multiforme	" " " "
A–4	?	192	Medulloblastoma	" " " "
A–5	?	192	Medulloblastoma	" " " "
1–3	F	193	Medulloblastoma	" " " "

methylcholanthrene had been completely exhibited and tumors had formed in all susceptible animals. Secondly, it seems clearly evident that the neoplastic process is considerably slower to appear in neurectodermal than in mesodermal tissues. Had the experiment continued longer this delay would have been more pronounced, since a number of the final gliomas were small tumors

which would have required several more weeks of growth before revealing their presence in living animals. Even with the abbreviated data available, however, it is demonstrated that the midpoint of the glioma series followed that of the sarcomas by approximately 50 days.

Within these two primary groups the number of individual types of tumors is too small to permit of statistical deductions.

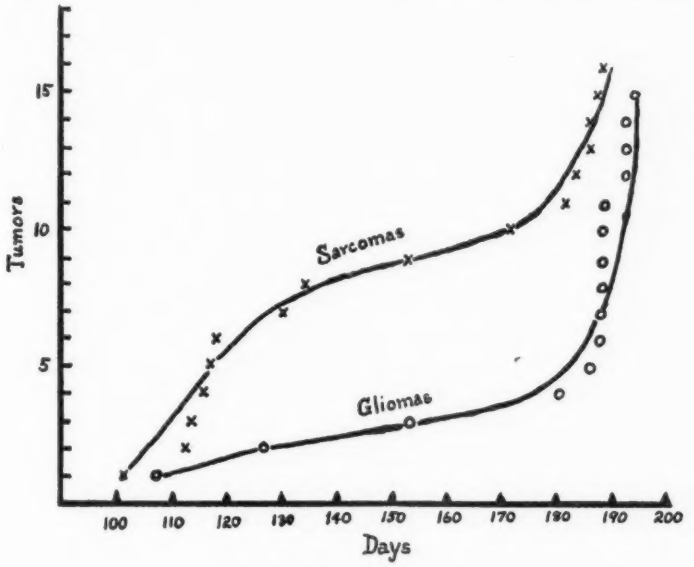

CHART 1

The reason for the first bulge in the sarcoma curve is unknown. It is only obscurely suggestive that all 3 rhabdomyosarcomas obtained were observed in this first period. Possibly striated muscle forms tumors infrequently but quite promptly.

PATHOLOGICAL OBSERVATIONS

As in the previous experiment,[1] the pellet containing the carcinogen was so placed as to maintain simultaneous contact with the skull, meninges and brain. The location of the pellet, and hence the site of application of the carcinogen, is readily demonstrable in sections. The pellet was found in all but 2 of the animals without tumors, and in the great majority of tumor-bearing mice.

In order to conserve tissue, blocks of the more bulky tumors were cut only deep enough to provide good representative sections, and in some the pellet doubtless lay beyond the plane of section. It is only in the small early tumors that the location of the pellet provided important information. In the large masses it was either deeply buried in pure tumor tissue or pushed at random to the periphery by the irregularly expanding growth. However, the pellet was always found within the cranial cavity even though the tumors frequently erupted through the trephine and formed the main mass externally.

The classification of the tumors produced is purely morphological, and for lack of any other basis it rests on the resemblance, in varying degree, of these induced tumors to human neoplasms. The difficulty and uncertainty are increased by the fact that all the tumors are rapidly growing tissues showing little differentiation toward adult structure. It is recognized that there exists therefore room for disagreement over the details of classification.

The primary partition of the entire series of tumors separates them into two groups: the sarcomas of mesodermal origin, and the gliomas of neurectodermal origin. Sometimes, as in the case of the rhabdomyosarcomas and the medulloblastomas, this division may confidently be made on the type cell of the tumor as displayed by routine stains. In the large group of undistinguished spindle cell tumors the most certain differentiation is based on the nature and arrangement of the intercellular substance. In undifferentiated and rapidly growing sarcomas, such as were produced in this experiment, the stroma consists only of very delicate reticulum. Masson's trichrome stain reveals the coarser parts of this stroma. Only a successful silver impregnation is adequate to display it completely. The success of a preparation is readily gauged by the clarity and intensity of impregnation of the pia-arachnoid and the stroma of the choroid plexus. By this means there is revealed a delicate but often abundant reticular stroma permeating the tumors of mesodermal origin. In contrast, the reticulum in gliomas is confined to the walls of the small and scanty vessels, as is also observed in human gliomas. The presence or absence of a reticular stroma invariably harmonized with the impression furnished by the type tumor cell, and thereby proved to be a most useful diagnostic criterion.

The Sarcoma Group

The tumors classified as rhabdomyosarcomas formed numerically the smallest group, and in time were the earliest to appear. Only 3 were observed, and all were recognized between 101 and 118 days. They all consist of hemispherical masses on the external surface of the skull, and were probably derived from the occipitofrontalis muscle. In all, the underlying skull is more or less eroded, and tumor is invading the diploe and elevating the dura for a short space at the edges of the trephine. In the 1st tumor observed there is a subdural plaque of tissue of considerable size compressing the brain but not invading the arachnoid. This animal alone was found in coma, the other 2 were recognized by the bulging of the scalp.

The tumor cells of these rhabdomyosarcomas are predominantly of bulky polygonal or spindle shape, with dense acidophilic cytoplasm and round to oval nuclei containing one to three large nucleoli. Tumor giant cells are present in varying numbers. Mitotic figures are numerous. The stroma consists of rather coarse and broken strands of reticulum permeating between tumor cells throughout the masses. Frequently cells seen in cross-section are encircled by a complete ring of reticulum. Vessels are scanty and necrosis appears only as small patches in the largest of the tumors.

The general appearance of these 3 tumors strongly suggests a myogenic origin, but proof in the form of cross-striated tumor cells was difficult to find. In an earlier paper Haagensen and Krehbiel [3] reported that they were unable to find cross-striated cells in any of 10 subcutaneous sarcomas of probable muscle origin produced by dibenzanthracene. Preexisting muscle fibers were discounted as far as possible. In 1 of the present tumors the phosphotungstic acid hematoxylin stain reveals a few slender, clearly cross-striated cells in the tumor invading the diploe and spreading beneath the skull from the trephine. From their position these cells are believed to be genuine tumor cells. A 2nd tumor of identical cell type contains a few such slender cross-striated cells deep in the mass. In contrast, no such striated cells were observed in the extracranial portions of the numerous other tumors of all types produced in this experiment. The third and bulkiest

tumor of this group contains many very large strap shaped giant cells with rows and terminal clusters of nuclei strongly resembling the giant cells seen in regenerating striated muscle. In the extracranial mass these cells might have been derived from preexisting muscle. In the large intracranial extension they are equally numerous and in this location they must unquestionably have been formed by differentiation of tumor cells (Fig. 1). With the phosphotungstic acid hematoxylin stain longitudinal myofibrils are clearly shown in these giant cells, but there are no definite cross-striations and no mitotic figures.

The tumors classified as fibrosarcomas, 12 in number, formed the most numerous type of tumor. In time of appearance they ranged from 113 to 187 days, but the majority were recognized about midway in the experiment. Six tumors were divided equally between the sexes, and in the remaining 6 the sex was not recorded. Five of these tumors evidently arose from the dural scar closing the trephine, and 7 were derived from intracranial tissues. Four were small and early masses discovered in routine examination at the end of the experiment. Eight, however, were grossly visible tumors of considerable size. Several primarily intracranial tumors had formed asymmetrical hour-glass shaped masses by erupting through the trephine.

In histological structure these fibrosarcomas constitute a quite homogeneous group. They are composed of slender, medium sized spindle cells closely packed in interwoven bundles. Their cytoplasm is finely granular and acidophilic and cell boundaries are rather indistinct. Nuclei are oval or round with a thin distinct membrane, one to six nucleoli and scattered chromatin grains. Mitoses are numerous in the majority of tumors. Between the individual cells there is a delicate but generally abundant web of reticulum fibers. Fine fibroglia fibrils are demonstrable on occasional tumor cells. Vessels are very scanty and small and there is practically no necrosis even in tissue in contact with the pellet. One tumor included with the fibrosarcomas is a small mass of very large and succulent cells originating at the zone of contact between the pellet and the skull. This may actually be an early osteogenic sarcoma, but the intercellular matrix consists entirely of fine and scanty collagen.

All fibrosarcomas of appreciable size actively invade and de-

stroy the adjacent skull, muscle and brain. Their relation to the adjoining brain is similar to that observed in human intracerebral metastases. These tumors of mesodermal origin appear to be immiscible with brain tissue. As a result the line between brain and advancing tumor is sharply demarcated (Fig. 2). This is in marked contrast to the gliomas which blend gradually into the neighboring brain. Extension of the fibrosarcoma often takes place along perivascular spaces. Portions of intervening brain isolated by this mode of growth show considerable fibrillary gliosis and subsequently disappear. The reticular stroma accompanies the sarcoma cells practically to their outermost extension, and furnishes an additional criterion by which to differentiate sarcomas from gliomas. Three fibrosarcomas were associated with gliomas. These multiple tumors will be discussed with the gliomas below.

The source of the small external fibrosarcomas is clearly the dura closing the trephine and lying in contact with the pellet. In one instance at least the underlying pia-arachnoid can be traced unaltered up to the edges of the pellet. Larger external tumors probably also arise from the dural scar.

The tissue of origin of the internal fibrosarcomas is less clear. Four structures, the meninges, the choroid stroma, vascular adventitia and the collagenous capsule about the pellet, are theoretically possible sources. As just noted, and also previously commented on,[1] whenever the meninges have been clearly demonstrated they have appeared to be entirely quiescent. Further, in none of the tumors has there been any histological structure even slightly resembling that characteristic of the meningioma. The choroid stroma may likewise be dismissed because the choroid plexus, though often in contact with the pellet and even embedded in tumor, has itself never displayed any suggestion of neoplastic reaction. By exclusion it seems that the internal fibrosarcomas are derived partly from vascular adventitia and partly from the capsule about the pellet. In one animal (No. 4–4) the latter origin is clearly demonstrated.

Two tumors considered to be leiomyosarcomas were separated with some uncertainty from the preceding group. Both arose deep in the brain substance, probably from smooth muscle in vessel walls. They invaded the adjacent brain and erupted through the trephine opening. One also extensively infiltrated the stroma of

the lateral choroid plexus. As in the fibrosarcomas, the line between tumor and brain though irregular is sharply demarcated. These tumors are composed of interlacing bundles of rather large and dense spindle cells, among which are scattered bulky irregular and club shaped giant cells. Nuclei are oval or irregular and mitoses are frequent. The tumor cell cytoplasm is dense, acidophilic and very finely granular. Myoglia fibrils are prominent in one tumor, and delicate and scarce in the other. Silver impregnation demonstrates a stroma of reticulum fibers parallel to or occasionally forming rings about tumor cells. This stroma is distinctly coarser and less abundant than in the fibrosarcomas. Vessels are very scanty. Necrosis appears only as a small area containing a few leukocytes and bordering on the pellet in one tumor.

The Glioma Group

Fifteen tumors appearing in 14 mice are classified as gliomas. This is but 2 less than the total number of sarcomas obtained. This fact is, however, of uncertain significance as there is evidence, stated above, that the experiment was terminated before carcinogenesis had been completed. As shown in Chart 1, the gliomas as a group appeared about 2 months later than the sarcomas. The termination of the experiment accordingly revealed a number of gliomas in early developmental stages. Such specimens proved most interesting because the pellets had not been displaced and relations distorted by massive tumor growth. In several instances it was possible to demonstrate the origin of special varieties of gliomas from specific types of brain tissue in contact with the pellet.

For lack of any more suitable scheme, the experimentally produced gliomas have been fitted into Bailey's classification [4] of human gliomas. As all the tumors are rapidly growing and poorly differentiated, their designation in some cases is more or less tentative. Following this classification there were identified 1 medulloepithelioma, 9 medulloblastomas and 5 glioblastoma multiforme.

The single medulloepithelioma was the earliest glioma produced. It was recognized on the 108th day as a large mass beneath the scalp. The overlying skin was intact and only slightly adherent

to the tumor. The point of origin could not be determined. A large growth destroyed the dorsal half of the thalamus and the overlying cortex, and erupted through the trephine to form an even larger subcutaneous mass. The tumor is composed of rather uniform epithelial-like cells of medium size (Figs. 3 and 4). Their cytoplasm is basophilic, finely granular or slightly foamy, and their nuclei large and round, with a heavy membrane and two to six large lumps of chromatin. Only a few cells are multinucleated. Mitoses are numerous. In most fields the cells are closely packed in cords and alveolar masses, but occasionally, chiefly in the external growth, there are small streaks and pools of coagulated albuminous fluid between the cells. Among the epithelial-like cells constituting the bulk of the tumor there are occasional more opaque cells with pale vesicular nuclei containing a single large nucleolus. Such cells are probably neuroblasts. The tumor cells produce no glia fibrils and the scanty strands of reticulum are clearly remnants derived from dura and vascular adventitia. The tumor blends into the adjacent brain with no evident line of demarcation. Vessels in the tumor are numerous. They are large, irregular thin walled channels, some of which are thrombosed. A small central core in the mass is necrotic.

The group of tumors classified as medulloblastomas constituted three-fifths of the total gliomas and were represented by 9 cases. They seem in general to have appeared slightly earlier and formed larger masses than did the glioblastoma multiforme. The histological structure of this group of tumors is quite uniform. They are composed of closely packed, small, round and oval, heavily chromatic nuclei embedded in a scanty and delicate fibrillary matrix that is not specifically stainable. Mitoses are very numerous. A narrow and ill-defined cytoplasmic rim is visible about the nuclei. Occasionally this is drawn out to one side, forming a small carrot shaped cell body. The cells are mostly growing in diffuse sheets, but in some fields in all the larger tumors they are arranged in small balls and rosettes, the center of which is filled by the fibrillary matrix (Fig. 6). No tumor giant cells are seen but occasional large astrocytic cells are present. The tumors blend without demarcation into the adjacent brain and spread along beneath the dura and meninges. In 3 animals the tumor had burst through the ependyma, and small masses of well pre-

served tissue appear to lie free in the ventricular fluid, or have formed small implants on the ventricular wall. Such behavior corresponds with the recognized tendency of human medulloblastomas to form widespread implants in the cerebrospinal pathway. A few minute patches of necrosis appear in the larger tumors. No glia fibrils are produced by the tumor cells and reticulum is limited to a few delicate strands from vessel walls and meninges.

The glioblastoma multiforme formed a smaller division of the gliomas represented by 5 tumors. These were the last of the gliomas to appear. They are less homogeneous in histological structure than the preceding group. All consist chiefly of medium sized spindle cells mingled with a lesser quantity of polygonal cells and a variable number of large tumor giant cells (Fig. 8). The cytoplasm of the smaller cells is poorly defined, and between them the background consists of frayed cell processes and a few glia fibrils. Mitoses are present but are less numerous than in the medulloblastomas. The 2 largest tumors invaded the dura and trephine. Growth within the ventricles and implants on the ependyma were not observed in this variety of glioma. In 1 tumor only there appeared small patches of necrosis with prominent palisading of cells about the edges. Vessels are small and few in number, and show no tendency to endothelial proliferation. The tumors of this group superficially resemble the sarcomas, but are differentiated from them by two well defined characteristics. They blend without clear demarcation into the adjacent brain and silver impregnation shows they contain no reticulum save a few strands in vessel walls and meninges.

Multiple Tumors

Three animals exhibited multiple simultaneous tumors arising at the points of contact between the pellet and the mesodermal and neurectodermal tissues. In each instance the external tumor is a fibrosarcoma originating in the trephine scar. The internal tumors are gliomas. In 1 the pellet lying obliquely in the cortex is surrounded by a tumor mantle having the structure of a medulloblastoma. In the 2nd the deep end of the pellet had crushed the fornix. At that point is found a small, actively growing glial tumor composed of spindle and polygonal cells and considered to be an early glioblastoma multiforme.

The 3rd and most complex multiple tumor is illustrated in Figure 9. The pellet is buried obliquely in the corona radiata and lateral ventricle. From its superficial end a large tumor mass structurally a fibrosarcoma erupts through the cortex and trephine and spreads beneath the scalp. About the medial end of the pellet, in the zone of contact with the gray matter of the cortex and the hippocampus, is a mantle of tumor morphologically a medulloblastoma (Fig. 10). A portion of this medulloblastoma spreads along the dura. Fragments of it float apparently free in the ventricular fluid, small implants are present on the wall of the opposite ventricle, and a large cast of such tissue fills the infundibular recess of the third ventricle. Laterally and deeply to the pellet, originating apparently in the coronal white matter and invading the thalamus, is a polymorphous and spindle cell glioma presenting the typical structure of a glioblastoma multiforme (Fig. 11). The line of demarcation between these 3 tumors is quite distinct, and the demonstration of reticulum by silver impregnation is especially valuable for differentiating the sarcomatous component of such multiple growths.

Discussion

These experimental gliomas, when viewed collectively, present several morphological features of considerable interest. The first is that only the more highly malignant varieties of gliomas are represented. This harmonizes with the now established general rule that the carcinogenic hydrocarbons produce exclusively tumors that are histologically undifferentiated and rapidly growing. The counterparts of the generally recognized benign tumors have not been observed as a local response to the hydrocarbons.

Because the experiment was terminated before its natural conclusion, a number of gliomas were obtained in early stages of development. In such growths the tumors had not yet distorted relations and displaced the pellet. These specimens clearly demonstrate that the gliomas classified as medulloblastomas were formed in the zone of contact between the pellet and the gray matter — commonly the cortex (Fig. 5), less often the hippocampus or thalamus. On the other hand, the polymorphous and spindle cell gliomas classified as glioblastoma multiforme appear to arise subcortically, at points where the pellet lay in contact

with the corona radiata or fornix (Fig. 7). This observation, if confirmed by further experiments, is of considerable theoretical significance. It seems to establish that the glioblastoma multiforme may be formed by anaplasia of white matter astrocytes.

The cellular origin of the medulloblastoma is less clear. It can scarcely be from cortical astrocytes for its structure bears no resemblance to gliomas of probable astrocytic origin. It also differs from the oligodendroglioma in cell type, rapid growth and the production of a fibrillary matrix. The tumor cells are very similar to early neuroblasts, but it cannot be determined if they are derived from anaplastic nerve cells in the gray matter, or from some unrecognized embryonic rest. The experiment at least proves that an adequate stimulus can evoke this variety of glioma from cortical gray matter remote from the natural site of predilection — the cerebellum.

Several non-neoplastic reactions in the brain are interesting enough to deserve brief notice. The most constant is a coarse spongy state, often marked, seen in the white matter external to the mild gliosis surrounding the pellet. A slighter degree of this change was occasionally noted about the dibenzanthracene pellets in the previous experiment. It is not evident whether this alteration is produced by trauma or by the irritative effect of the carcinogen. It is not accompanied by gliosis, and bears no apparent relation to the development of tumors. The absence of recognizable intermediate stages between non-reacting and tumor-bearing brains is noteworthy. Once started, the neoplastic process apparently developed with great rapidity and obliterated transitional changes, if any, in even the earliest tumors observed.

The complete passivity of the ependyma is a striking and constant feature. In the majority of specimens the pellet had penetrated the ventricle, yet the ependyma was merely slightly flattened and everted. Tumors of several sorts ruptured into the ventricles. Ependymal cells, recognizable by the clump of blepharoplasts in their cytoplasm, stretched over the advancing edge of such tumors and disappeared. A few were buried in the margin of the growth. In 1 animal the interventricular foramen had been sealed, probably by trauma. A marked unilateral hydrocephalus developed. The animal was killed on the 145th day on suspicion of tumor because of moderate bulging of the head. The pellet

was found lying free in the greatly dilated lateral ventricle. The ependyma was complete though much stretched and flattened, and showed no trace of hyperplasia or tumor formation. The refractory nature of the ventricular lining is also confirmed by the negative results of numerous intracisternal injections of carcinogens reported by Seligman and Shear.[2]

As a rule, the choroid plexus seems to be as unresponsive as the ependyma to the presence of the carcinogen. In over half the animals without tumors the choroid plexus lay in contact with the pellet. The plexus was often flattened and displaced, but neither epithelium nor stroma shows more than deformity from mechanical pressure. In animals with tumors the choroid was also inactive, even though in some it was either invaded by or buried in tumor tissue. Morphological alteration was observed only twice. In these cases a medulloblastoma had produced mild obstructive hydrocephalus. The choroidal epithelium in the dilated ventricles is somewhat hyperplastic, with enlarged and succulent cells, some of which appear to be discharging vacuoles of secretion. There is no suggestion of tumor formation, and the change is probably a result of the ventricular obstruction.

Non-specific inflammatory reactions about the operative field were fairly common, but were seemingly without influence on the development of tumors. In 14 animals they were of sufficient degree to be noted in the protocols, but were insignificant save in 1 animal which died of meningitis and an abscess 31 days after operation.

Necrosis was uncommon and limited to a few small patches at a distance from the pellet. In contrast, more or less marked necrosis is mentioned in half the tumors described by Seligman and Shear.[2] As they used pellets of pure methylcholanthrene, some of the necrosis may have been the result of the high concentration of the very potent carcinogen. In earlier studies [5, 6] Shear noted that subcutaneous injection of concentrated doses of potent hydrocarbons caused considerable necrosis and ulceration without correspondingly accelerating or increasing the production of tumors. In the brain it is likely that such tissue destruction actually retards tumor development as the very prolonged latent period in their recent experiment suggests. Their earliest tumor required for its formation 227 days, a month more than the total

duration of the present experiment. The optimum local concentration of methylcholanthrene may be in the range of 10 per cent, and excessive concentration may produce undesirable side reactions that to some degree interfere with tumor formation.

A complete autopsy was performed on all animals in this study but only 1 incidental tumor was discovered. In a male dying 92 days after operation there was found a sharply defined hemispherical gray mass 3 mm. in diameter immediately beneath the pleura of the lower lobe of the left lung. Microscopically this tumor is a benign papillary adenoma composed of a circumscribed mass of delicate vascular fibrous fronds covered by a single layer of uniform low columnar epithelial cells without cilia or evidence of secretion. No mitoses are present, and there is no invasive growth or metastases. The immediate cause of death was purulent bronchitis and bronchopneumonia.

As indicated in Table I, objective symptoms caused by these experimental intracranial tumors were not impressive. This is probably a consequence of the relatively simple functional specialization in the mouse brain. Slightly more than half the animals presented no symptoms. Their tumors, usually early, were discovered in the routine examination at the end of the experiment. The external tumors and also the majority of internal tumors were recognized by the bulging of the scalp. Careful watch was kept for this sign, and as a result no tumor-bearing animal was allowed to die naturally and the tissue deteriorate. The bulk of an intracranial mass that could be tolerated without obvious symptoms was sometimes surprising. One such animal presented an intracranial fibrosarcoma estimated to equal in size the volume of the normal brain. The calvarium was thinned and the sutures separated, yet the tumor was diagnosed during life by the gross bulging and the animal seemed only somewhat sluggish. As the limit of tolerance was approached, the larger masses produced a lethargy deepening into a coma that appeared to last at least for several hours. Two animals showed a unilateral extensor spasticity causing them to move in circles toward the operated side. One of these, the bearer of the 3 tumors described above, also appeared to be blind. One animal with a large internal fibrosarcoma, while being examined, had a generalized convulsion with opisthotonos and flexion away from the operated side.

Summary and Conclusions

Cholesterol pellets containing 10 per cent of methylcholanthrene were successfuly implanted into the brains of 99 stock albino mice.

The first tumor appeared on the 101st day, and the experiment was terminated after 193 days. Eighty-seven animals survived into the tumor-bearing period, and all of them not developing tumors lived to the end of the experiment.

A total of 32 tumors was observed in 28 of the 87 surviving animals. Seventeen of the tumors were classified as sarcomas of mesodermal origin, and 15 were gliomas. Three animals presented more than one type of tumor.

Evidence is presented that the experiment was terminated before all possible tumors had been secured. It is also shown that under approximately the same conditions nervous tissue responds to the carcinogenic stimulus as frequently, but definitely more slowly than the neighboring mesodermal tissues. The latent period for gliomas averaged about 50 days longer than that for sarcomas.

The criteria for classification and the histological structure of the different varieties of tumors have been described in some detail. Sarcomas consisted of 3 of striated muscle, 12 of fibroblast and 2 of smooth muscle origin. Gliomas consisted of 1 medulloepithelioma, 9 medulloblastomas and 5 glioblastoma multiforme. All tumors produced were rapidly growing and poorly differentiated neoplasms.

From early tumors of small size morphological evidence has been produced to indicate that the glioblastoma multiforme was derived from the cerebral white matter, probably by neoplastic transformation of astrocytes. Similar observations suggested that the medulloblastoma arose from the gray matter, possibly by anaplasia of nerve cells since the tumor cells resembled neuroblasts.

Within the duration of the experiment no suggestion of neoplastic reaction was observed in the ependyma, the pia-arachnoid or the choroid plexus, though each was exposed to the carcinogen in many animals.

The tissue surrounding the pellets was strikingly free of necrosis and non-specific inflammation. The 10 per cent pellets of methylcholanthrene employed probably approximated the optimum

carcinogenic concentration. Higher concentrations, by producing local necrosis, may actually retard tumor formation.

NOTE: The author gratefully acknowledges the assistance of a former student, Mr. J. C. Cope, who designed and made the pellet injector, and of Mr. J. W. McBride, custodian of animals, whose vigilance in detecting every tumor-bearing animal before its death made possible the obtaining of perfectly preserved tissue for microscopic preparations.

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DESCRIPTION OF PLATES

PLATE 134

FIG. 1. Intracranial portion of a rhabdomyosarcoma (No. 18–3) showing numerous giant cells. The compressed brain appears along the lower margin. Hematoxylin-eosin stain. × 100.

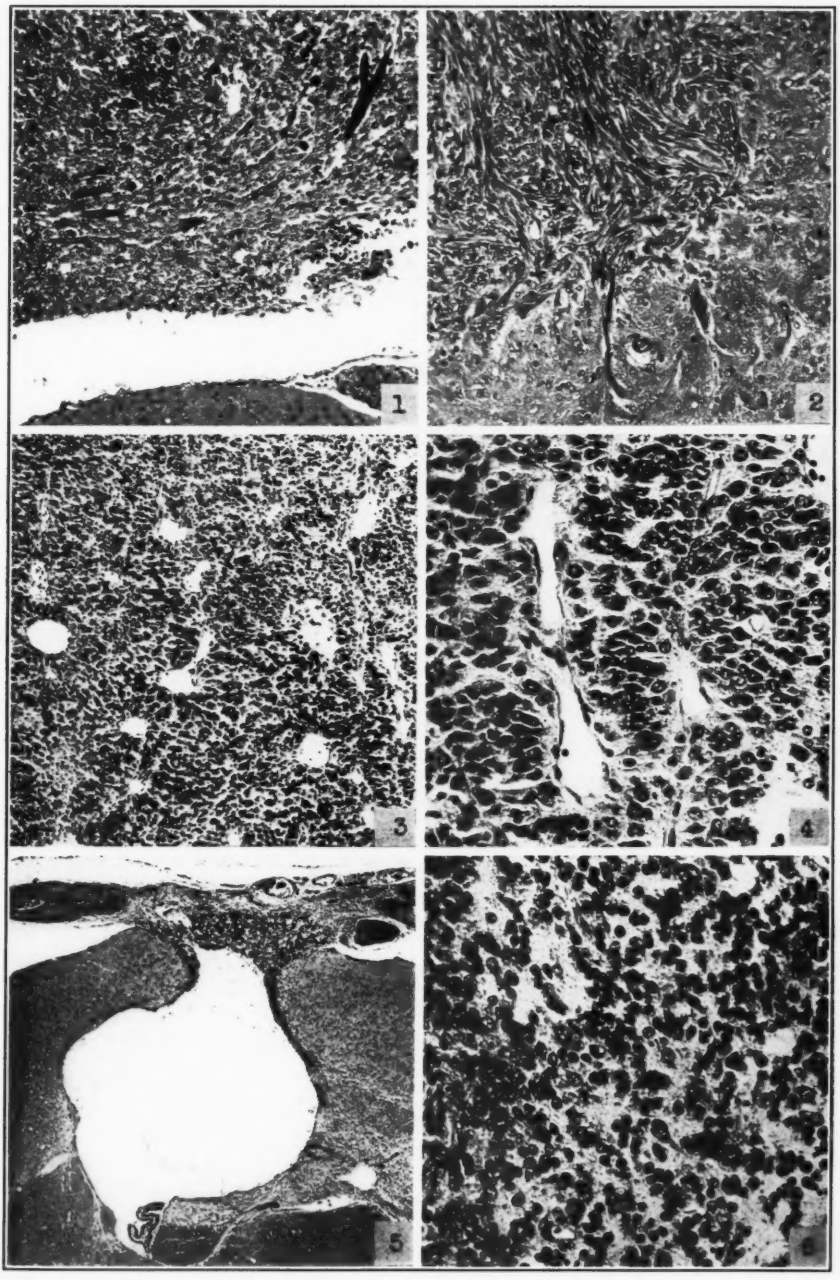
FIG. 2. Edge of an intracranial fibrosarcoma (No. 7–3) showing a sharply defined margin and perivascular extension. Hematoxylin-eosin stain. × 135.

FIG. 3. Medulloepithelioma (No. 12–1) showing the general arrangement of tumor cells in cords. Phosphotungstic acid hematoxylin stain. × 80.

FIG. 4. Medulloepithelioma (No. 12–1) showing the undifferentiated, epithelial-like character of the cells and their arrangement in cords between the thin walled blood channels. Phosphotungstic acid hematoxylin stain. × 270.

FIG. 5. Early medulloblastoma (No. B–8) showing the origin of the tumor from the cortical gray matter in contact with the superficial surface of the pellet. The position of the pellet, dissolved in embedding, is marked by the large empty cavity. Masson's trichrome method. × 30.

FIG. 6. Histological structure of the medulloblastoma shown in Figure 5. Clumps and poorly formed rosettes of small round nuclei with a scarcely visible cytoplasmic rim are embedded in a delicate fibrillary matrix. Masson's trichrome method. × 270.



Peers Response of Central Nervous System. II

PLATE 135

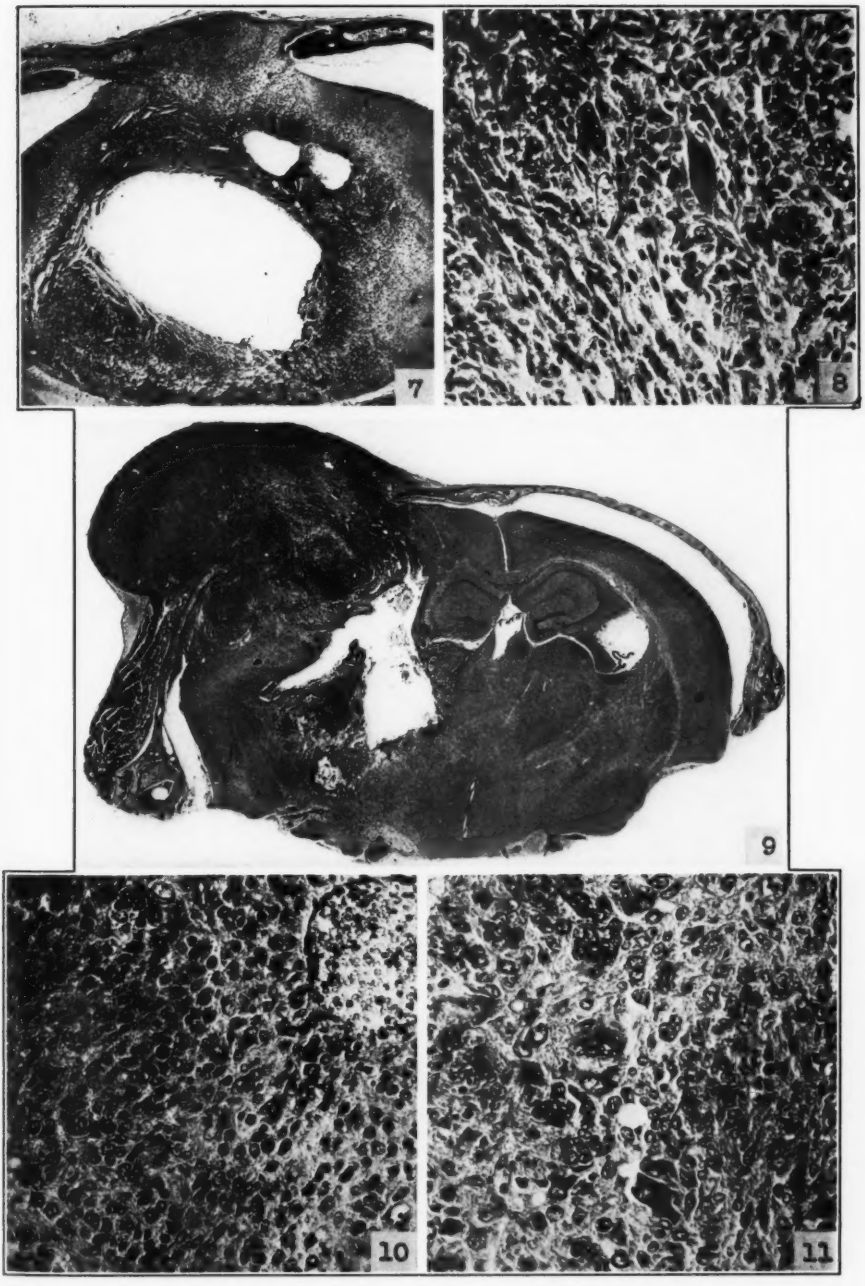
FIG. 7. Early glioblastoma multiforme (No. A–3) showing the origin of the tumor about a pellet buried deeply in the subcortical white matter. Phosphotungstic acid hematoxylin stain. × 19.

FIG. 8. Histological structure of the tumor illustrated in Figure 7 showing a polymorphous cell type with tumor giant cells and a mitosis to the right of the center. Phosphotungstic acid hematoxylin stain. × 270.

FIG. 9. Mouse (No. 8–4) bearing three types of tumor. The large external mass is a fibrosarcoma. In the cortex about its deep edge is a medulloblastoma. The triangular tumor in the thalamus to the left of the pellet is a glioblastoma multiforme. Tumor implants are barely visible in the opposite ventricle and infundibular recess. Phosphotungstic acid hematoxylin stain. × 10.

FIG. 10. Medulloblastoma from the mass seen in Figure 9. The field was selected from the medial edge of the cerebral cortex. Phosphotungstic acid hematoxylin stain. × 270.

FIG. 11. Glioblastoma multiforme from the mass in Figure 9. The field was selected from the triangular tumor to the left of the pellet. Phosphotungstic acid hematoxylin stain. × 270.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THE PATHOLOGY AND PATHOGENESIS OF MAMMARY TUMORS OCCURRING SPONTANEOUSLY IN THE ALBANY STRAIN OF RATS *

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Spontaneous mammary tumors in rats occur from time to time in almost any colony, but the appearance of such newgrowths in fairly large numbers is rare. Recently Bryan, Klinck and Wolfe [1] published a report on the Albany or A–S strain of rats, in the females of which breast tumors were appearing with unusual frequency. This strain had been started in 1930 as a breeding colony and from that time on careful genealogical records of the females were available. Although matings of closely related animals were not planned or consciously practised, inbreeding presumably occurred with considerable frequency and the strain must have become rather homogeneous. Breast tumors were first noticed in a few animals in 1934, but from then on they appeared with increasing frequency. Little by little, too, the fertility of the colony became reduced and the entire group of animals was taken over in late 1936 for careful study.

For nearly 3 years now the A–S strain has been under constant observation and by close inbreeding an attempt is being made to produce one or more high tumor lines. It is yet too early to say whether or not this attempt will be successful. However, at least three, and possibly five lines have been obtained in which there are definite tendencies in the direction of more frequent spontaneous tumor development.

In several respects these rats are unusual, the animals revealing a number of abnormalities which now appear to be characteristic of the strain. The most important of these, aside from tumor development, are abnormal changes in the sex cycles in the females and diminished fertility in both males and females,[2] prolonged

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gestation and spontaneous fetal resorption in a high percentage of the females,[3] and definitely deficient growth of these animals when they are compared with others of a normal strain.[4] Studies made so far, in addition to the tentative results of several investigations which are not yet complete, indicate that the abnormalities observed are endocrine in character and strongly suggest that they are secondary to deficiencies in the anterior lobe of the pituitary gland.[1]

The most striking obvious abnormality is the relatively high rate at which the spontaneous mammary tumors have appeared in females (and of late, incidentally, in an occasional male). In the small group of 51 females which were originally studied [1] the tumor incidence was 50.9 per cent. That high percentage has not been duplicated in the expanded colony of today, but among 1827 females which have reached the age of 5 or more months, 186 mammary tumors and 11 small sclerotic lesions which we consider to be very early tumors have appeared.

The part, if any, which the observed endocrine disturbances play in bringing about tumor development is still the subject of study. It may be said, however, that the endocrine changes which are manifested by alterations in the estrus cycles in the females of this strain are not unlike those which occur quite commonly in very old rats of other strains but which, in our animals, appear early in life. The process of ageing in the A–S rats thus seems to begin at an earlier time than in other rats. This suggests that the mammary newgrowths which, when found in occasional rats of other colonies, usually occur in very old animals, may be more numerous in younger rats of this strain, sometimes at the age of 5 to 10 months, because of the premature onset of the changes characteristic of old age. It is easier for the A–S animals, so to speak, to live to reach the physiological period of life at which tumors most often appear.

To date, animals of this colony, since they came under observation and including the original group, have numbered 6055, of which 3192 were females. So far only 1827 of the latter have reached the age of 5 or more months, the earliest age at which mammary tumors have yet appeared. Of this group 436 have been stock animals, that is, the offspring of rats of the A–S strain but not of specific lines, while 1391 have been members of 36 different

specific lines in which the females have been bred usually to their brothers or, if these were non-fertile, to their fathers, uncles or cousins. Sixteen of these lines have now been discontinued. In one line, 147, in which the ancestor female was one of the original group of tumor bearers, 18 tumors have already appeared among 80 female offspring which have reached the age of 5 or more months. In this line 17 females are now in the fourth generation. In two other lines the incidence of tumors also appears to be increasing but not at quite as rapid a rate. Among the stock animals mammary neoplasms have appeared with considerable frequency and in Table I, in which are recorded all the tumors which have appeared up to Aug. 1, 1939, the figures include females in both the stock and the closely inbred lines.

The total number of 6055 rats has been reached in spite of the fact that many animals were sterile while others manifested varying degrees of diminishing fertility. The reproductive deficiencies, as Wolfe, Burack and Wright [2] have shown, are due chiefly to gonadal abnormalities in both males and females. Moreover, Burack, Wolfe and Wright [3] have reported a diminution in the number of litters dropped by the fertile females. These have averaged only 2 per female. These workers found, too, that as a result of partial or complete fetal resorption, which occurs at the abnormally high rate of 25 per cent of all pregnancies, the number of living young which were delivered was well below average, varying from 2 to 5 or 6 per litter and averaging only 4. The reproductive performance of the A–S rats is therefore very poor.

The tumors which have developed in the breasts of female rats of this strain were practically all benign growths of fibroepithelial type, although 1 carcinoma which metastasized, 1 questionable fibrosarcoma, and a few very cellular and sometimes moderately anaplastic adenomas or cystadenomas were found. All were quite readily detectable on gross examination of the animals and some grew to great size. In addition, in 11 cases small, indefinite, vaguely nodular masses which appeared to be the early stage of tumor development were discovered at the time of autopsy of non-tumor-bearing animals. Similar lesions were found histologically in the mammary tissues of some animals with tumors even though, either because they were overshadowed in importance by the larger growth or were too indefinite to attract attention, they

were not generally noted clinically or on gross examination. Although we do not wish to concern ourselves in this report with tumors in other parts of the body, it may be said in passing that

TABLE I

Ages in Months at which Mammary Tumors First Appeared

Age in months	Total number of females which have reached given age	Total number of females which developed tumors at given age
5	1827	3
6	1549	0
7	1364	3
8	1262	1
9	1159	10
10	1018	8
11	895	7
12	782	11
13	687	11
14	616	15
15	554	13
16	477	14
17	404	14
18	327	10
19	262	12
20	188	12
21	143	7
22	94	8
23	70	3
24	54	2
25	38	3
26	22	4
27	18	0
28	14	0
29	7	0
30	4	0
31	1	0
32	1	0
		171
	Tumors for which age of onset is not known	15
	Total	186

The figures in the first column indicate ages in months. In the second column the figures represent the total number of female rats, which throughout the period of our study from 1936 to date were living at the given age. This total in each case includes, first the rats that had reached that particular age and then died, second those rats that survived and continued to live on to older ages, and, third, those rats that are now alive at the different ages.

The last column of figures indicates the number of female rats, from the beginning of our study, which showed the first evidence of a tumor at each particular age. This number is not a cumulative one. It lists only the rats that were first discovered to have a mammary tumor when they reached the particular age.

benign or malignant non-mammary newgrowths occurred occasionally in rats of both sexes.

In this paper we wish to record the results of a study of 141 mammary tumors out of the total of 186. Forty-five rats with

tumors are still alive and as yet tissue from these newgrowths is not available for histological study. Nevertheless, since they have all the gross characteristics of the lesions which in other rats have been proved to be newgrowths, they are considered to be neoplastic. In addition to the 141 tumors we have also carefully studied the mammary lesions from the rats which had the small, indefinite but readily palpable areas of thickening which did not meet the histological requirements for a diagnosis of true neoplasia, but which we are inclined to interpret as the earliest stage of tumor development. The latter lesions we have not grouped as tumors.

Location of the Tumors: In general the newgrowths have developed within the confines of the breast tissue, especially at the caudad and cephalad portions of the mammary lines. They have been about equally divided between the right and left sides. Ninety-four tumors occurred in the axillary regions while 52 were present near or in the groins. The balance, except for a few in which the site of the tumor was not recorded, originated chiefly in the regions of the second, third or fifth mammary glands on either the right or the left side. In a few cases, those in which very large tumors had developed before the animals came under our observation, it was difficult to determine accurately the site of the original growth. Ectopic breast tumors were not found.

In general the newgrowths observed so far were single but in 8 rats 2 tumors were present in each animal (Fig. 1). In none of the animals were there more than 2. In several cases the 2 tumors, incidentally, showed different rates of growth as well as gross and histological variations which made them appear to be quite separate and unrelated neoplasms.

Size of the Tumors: The tumors which have been examined thus far have varied considerably in size. The more recent newgrowths of course are still small, varying from a few millimeters to 3 or 4 cm. in greatest dimension. When the growths were not excised, however, and when the animal continued to live for a long time with the enlarging mass, the tumors sometimes grew to very considerable proportions, reaching sizes in which they measured 10 or 11 cm. in greatest dimension and weighed 150 to 200 or more gm. In rare cases they even weighed more than the host rat. In Rat A–S 35, for example, the growth at the time of

autopsy measured 10.5 by 8 by 6.5 cm. in size and weighed 200 gm., almost as much as the host. In another rat (A–S 9), the tumor weighed 205 gm. while the host animal, after its removal, weighed only 200 gm. In general, however, 75 per cent of the mammary newgrowths measured 3 cm. or less in greatest dimension while only 15 per cent measured 6 cm. or more.

Gross Characteristics of the Tumors: The tumors which have been studied histologically were all discrete localized masses which could usually be seen with the naked eye and could always be palpated through the skin as definite circumscribed nodules. They were nearly always approximately spherical or ovoid in shape but were sometimes flattened masses 1 or 2 cm. in diameter but only a few millimeters in thickness. Generally these growths were freely movable and not attached to the skin unless, as occasionally happened when one of the larger growths became ulcerated as a result of pressure necrosis or injury, inflammatory changes bound the skin and the newgrowth firmly together. The largest of the newgrowths were commonly distinctly lobulated, from several to many smooth rounded convolutions being palpable even through the skin.

When the tumors were exposed either surgically or at autopsy the majority of them were found to be separate, circumscribed encapsulated masses which stood out prominently from the surrounding tissues. Even the known carcinoma and the other neoplasms which appeared malignant but which did not metastasize were discrete, localized, and had enveloping membranes. The larger growths often showed lobulations of varying size. In some of the earliest and smallest neoplasms encapsulation and lobulation were not always detectable, but generally these characteristics seemed to be acquired at a rather early stage in the development of the growth.

When the tumors were removed they usually could be shelled out quite easily from the surrounding mammary tissues to which they were attached not only by strands of delicate fibrous tissue but also to some extent by the blood vessels which entered them. Often these vessels were so small as to be insignificant but in the more massive growths they were usually large and prominent. In some cases (Fig. 2) they consisted of a single artery and its accompanying veins, which formed a sort of pedicle for the encap-

sulated neoplasm. More commonly several vessels entered the growth at various points about its periphery.

The capsules surrounding the tumors were thin, delicate, rather elastic membranes which were only fragmentary in the smaller growths but which, in the larger ones, were often extensive sheets of tense, semitransparent areolar connective tissue. If the tumor was lobulated the capsule presented surface irregularities which conformed to the lobulations and often gave off thin membranous septums which dipped deeply into the depressions between the lobules. In the very small tumors, because their outlines were not clear-cut and encapsulation was lacking, the growths were not well defined and were usually removed with a small amount of the surrounding mammary tissue attached.

When cut open the stroma and parenchyma of the larger growths tended to burst through the capsular incision or to bulge beyond their delimiting membranes after the growth had been cut across. Even in the same tumor the cut surfaces generally varied in character, some parts being dense and fibrous, others smooth, velvety and soft, while still others were glandular and compressible. In some newgrowths all the tissues were dense, firm, and appeared fibromatous. More rarely they were edematous and resembled a myxoma. Frequently they had a mixed fibroglandular appearance, neither type of tissue predominating. Still others were composed of an abundance of soft, moderately compressible acinar tissue, sometimes cystic and without much stroma. In the larger growths foci of degeneration or necrosis, with or without hemorrhage, were not uncommon. Occasionally a thin whitish secretion could be expressed from the cut surfaces, indicating secretory activity within the tumor. This was usually found when the rat was pregnant or lactating at the time the tumor was removed. Between the two extremes of fibrous and glandular types of growth there were all variations of combination of the glandular and stromal tissues, even in the same tumor. It may be stated, however, that considering the tumors as a whole those in which fibrous tissue predominated were by far the larger group.

Because we feel that the focal areas of mammary thickening found in 11 animals without tumors represent very early stages of tumor development, we include here a brief description of these

lesions as they were observed grossly. They were minute, often barely palpable foci. They had no definite outlines, were not encapsulated, and their borders seemed to fade imperceptibly into the adjacent mammary tissue. In some cases these foci were quite firm and dense, as if they contained an excessive amount of connective tissue; in others they had a smooth rubbery feel, as though composed of a mixture of fat, glandular tissue and fibrous stroma. Occasionally, too, they were unduly soft and compressible, even though only vaguely outlined, and gave off a thin whitish fluid from their cut surfaces. Sometimes they seemed to fan outward indefinitely into the peripheral breast tissue. That they were very definite aberrations from the normal arrangement of breast tissue was evident, even though they were not considered to be typical tumors. It should be noted that similar foci were found histologically in the mammary tissue of a number of rats with tumors, but practically always they had passed unnoticed on gross examination of the breasts.

Histological Structure of the Tumors: The majority of the new-growths studied were fibroepithelial in character. These include the group of tumors called by various names — adenofibroma, intracanalicular adenofibroma, pericanalicular adenofibroma, periductal fibroma, fibroadenoma, and so on. Ninety of them were primarily fibrous, while 32 were composed chiefly of glandular elements. However, 10 histologically pure fibromas, 11 typical adenomas, 5 cystadenomas and 1 carcinoma were also found in the total of 149 tumors from the 141 animals.

In the fibroepithelial tumors there was a great variation in the proportion of glandular tissue to stroma and in the histological character of the growths. In the majority fibrous tissue formed the bulk of the growth and the ducts and their terminal branchings varied from minute, contracted, non-active gland-like spaces to somewhat larger, more abundant acini which were often lined by large epithelial cells with varying numbers of vacuoles in their cytoplasm. The character of the epithelium tended to resemble more or less closely that present in the normal breast tissue of the same animal and there was evidence that in the majority of the growths the secretory epithelium responded to hormonal stimuli in somewhat the same manner as did the normal acinar tissue. Occasionally a form of growth not unlike that in the intracanalicu-

lar papillary adenofibroma of the human breast was found but such growth was relatively uncommon.

In other cases glandular tissue was more abundant than stroma. Here, too, the character of the secretory epithelium differed in different tumors, varying from the resting state to that in which moderate to marked activity was present and tending to resemble the normal mammary epithelium in non-neoplastic portions of the breast. All of these growths, in spite of their differing histological make-up, must be considered as essentially the same. The observed differences appeared to be due to some variation in the manner in which the affected portion of the breast responded to some stimulus, probably hormonal in character but as yet unknown.

In Figures 3 and 4 are shown typical examples of fibroepithelial newgrowths in which there is generalized increase of connective tissue in the tumor, that is, proliferation of fibrous tissue not only in the lobules themselves but also between them, with very slight increase in glandular tissue. Figures 5 and 6 show higher power views of this type of growth. The latter figure shows what we consider one method by which these tumors increase in size, that is, by the addition to the already growing tumor of previously focally sclerosed portions of adjacent mammary tissue.

Figure 7 illustrates a tumor of the same general type as those already described but in which glandular structures are more abundant than fibrous stroma. There was also evidence of marked secretory activity in this tumor, as shown in Figure 8. This particular neoplasm came from a pregnant rat which was already past term at the time of death.

In 10 cases tumors which consisted almost entirely of connective tissue were found (Figs. 9 and 10). Occasionally, particularly at the peripheral portions of these tumors, a few duct-like or glandular structures could be made out. Elsewhere the growth consisted of fibrous tissue which in some tumors was extremely dense, almost acellular and hyaline, but which in others was younger and more active appearing. In the latter moderate numbers of proliferating fibroblasts, as evidenced by cells in mitosis (Fig. 10) were usually found. When stained by Mallory's aniline blue or the phosphotungstic acid hematoxylin method, the abun-

dant fibrils which were present gave a reaction characteristic of collagen.

All of the above tumors, including the purely fibrous ones, must in our opinion be grouped together as newgrowths of fibroepithelial character differing merely in the degree of stroma or gland proliferation. Out of 149 tumors examined histologically, 132 fell into this group.

Eleven of the tumors were pure adenomas (Figs. 11 and 12), while five were cystadenomas which showed varying degrees of growth activity (Figs. 13 to 16 inclusive). In all of these newgrowths there was definite and usually atypical glandular hyperplasia so that, as shown in Figures 11 and 12, encapsulated glandular structures practically without fibrous stroma were often formed. In those tumors which were cystic there was evidence of marked epithelial proliferation, frequently with the formation of papillary projections. The cells were large, with fairly abundant cytoplasm and small, oval chromatic nuclei which were deeply situated. Mitotic figures in some cases, as in Figure 16, were abundant. From a histological point of view 2 or 3 of these neoplasms could well have been considered malignant but since we adopted as a necessary requirement for such a diagnosis the presence of remote metastases, which in these cases were not found, the tumors have not been listed as carcinomas. That they were at least precancerous growths we do not doubt.

Only 1 of the tumors was malignant and produced secondary metastases in the lungs. This was a primary mammary carcinoma which exhibited a varied histological picture. It has been described at length elsewhere [1] and will therefore not be considered in detail here.

From a histological standpoint the changes which were found in the 11 rats which showed focal thickenings in the mammary glands were of two general types. In the first there was a diffuse increase of interstitial fibrous tissue which involved primarily a small lobe of the breast, as in Figure 17 where the process appeared to be in its earliest stage, Figure 18 where the fibrous tissue was more abundant, or in Figure 19 where actual early tumor formation was evident. In the second type the significant change was a very localized increase of connective tissue which occurred quite specifically around groups of small ductules and

their end buds or acini, usually without early involvement of the interlobular fibrous tissue, as shown in Figures 20 to 23 inclusive. Even here the process still tended to affect only lobules which were connected with a single small lobar or interlobar duct.

In the more diffuse form the interlobular fibrosis appeared to progress slowly and the normal fat tissue became gradually replaced. Whether or not fat cells themselves actually took part in laying down collagen fibrils, we do not know. While the interlobular connective tissue was increasing there was also evidence of proliferative activity in the fibrous tissue of the lobules themselves, with gradual separation of the epithelial elements. The enlarging mass then expanded gradually as the new tissue increased in amount, forming a small tumor which ultimately acquired a capsule by compression of the adjacent normal stroma.

In the more localized form the intralobular fibrosis was found first in a single small focus, as in Figure 20. Here the areolar connective tissue which was present around and between the ductules and their end and lateral buds or acini, increased in amount and became thickened and dense. It surrounded, separated, and often tended to compress somewhat, the epithelial structures. Soon minute foci of lobular sclerosis, resembling miliary adenofibromas, resulted, as shown in Figures 21, 22 and 23. These were at first separated by normal mammary fat but little by little even the fat became replaced by fibrous tissue until a small, dense, but typical fibroepithelial tumor was formed (Fig. 22).

In many rats which had typical mammary tumors small foci showing the same sequence of events could be found in sections from parts of the breast which were remote from the obvious newgrowth. These focal, non-tumorous fibrotic changes are considered to be the earliest stages of tumor development.

Discussion

We have described the general gross and histological features of 149 mammary tumors which appeared up to Aug. 1, 1939, in 141 rats of the A–S strain. In addition we have given a description of certain focal sclerotic lesions which are considered to be the earliest stages in the development of these tumors. The great majority of the newgrowths, 132 in all, were typical of the new-

growths which, in the human breast at least, go under such names as adenofibroma, fibroadenoma, periductal fibroma, and other similar designations. Tumors of this type occur occasionally in old female rats of other strains but in animals of the A–S strain they have not only occurred with sufficient frequency to be unusual but have appeared at earlier ages. It is not our purpose in this discussion to consider the cause of the development of these tumors since our studies to date have not revealed the specific reason for their origin. In the human subject the growths have been considered by Ingleby [5] to be caused by a local irregularity of the cyclic breast changes which occur normally. These irregularities result in proliferation of the mammary tissues, followed by foci of incomplete regression or no regression at all. The localized excess of growth over regression, granting that it takes place regularly and over a long period, Ingleby believes to be the cause of tumor development. Certainly in the female rats of this strain there is every evidence that there is abnormal activity of the ovary which in turn is probably secondary to a dysfunction of the anterior lobe of the pituitary gland. It seems reasonable to assume, therefore, that these endocrine irregularities, with their consequent abnormal stimulation of the breast tissues, could well be associated, to some degree at least, with tumor development in the mammary gland. However, it also seems probable that genetic factors play some part in the causation of the newgrowths.

The state of development of the acinar tissue in any given tumor apparently depended, in some measure at least, on the same influences that acted on normal mammary epithelium. While no attempt is made in this paper to relate the specific changes that occurred in the epithelium of the tumors to phases in the estrus cycle or to abnormalities in the cycle itself, it may be said briefly that, as Ingleby [5] has contended in the case of fibroadenomas of the human breast, hormonal substances evidently could affect glandular tissue in the tumors just as they affected similar tissue in the normal portions of the breast. Thus in animals whose uninvolved mammary tissue showed the proliferative epithelial changes characteristic of the effect of the follicular hormone, somewhat similar changes were found in the glandular structures of the tumor. Likewise, if the hyperplasia characteristic of late

pregnancy was found in the normal portions of the breast, similar hyperplastic changes were found in the adenofibroma, even to the point where actual secretion occurred (Fig. 8). It is believed that, to some degree at least, the differences between the cells in the epithelial structures in the tumors of different animals, as well as the great variations in the proportion of acini to stroma in different newgrowths, were due to variations in the hormonal stimulation of the tissues themselves.

Several investigators have noted measurable, even large amounts of estrin in benign adenofibromas of the breast. Lewis and Geschickter [6] found this hormone in high concentration in a fibroadenoma of the human breast. Heiman and Krehbiel,[7] in their studies of growing transplants of mammary fibroadenomas, found fairly large amounts of estrogenic substance, especially in old rats. They considered that the hormone became fixed in the tumor tissue. Mohs,[8] on the other hand, found no demonstrable estrin in adenofibromas of the rat mammary gland and Frank, Goldberger, Salmon and Friedman [9] warned that the finding of the estrogenic factor in abnormal tissues should not be considered significant unless control studies of normal tissues showed no widespread distribution of this substance. Although our own experience is as yet very limited, preliminary bio-assays of tumors from some of the A–S rats indicated no detectable amounts of estrin in quantities of tumor tissue up to 25 gm.

Preliminary studies of the effect of prolonged administration of estrin to rats with spontaneous mammary adenofibromas have indicated that the most usual result is a proliferation of the glandular tissue along with characteristic changes in the epithelial cells themselves. In some cases the tumor grew more rapidly than usual during the period of estrin injections; in other cases this did not happen. To date, in a series of about 30 animals, no benign adenofibromas have been rendered malignant by prolonged estrin injection. Briefly, the histological changes observed were slight to moderate proliferation of the smaller ducts and their end buds to form more acinar tissue, marked vacuolization of the epithelial cells lining the acini, and sometimes, but not always, an apparent diminution in the amount of fibrous stroma. The apparent diminution in fibrous tissue is believed to have resulted, in part at least, from compression by the increased amount of

glandular tissue which was formed. The administration of estrin, as noted by Murphy, Schilling, and Emge [10] in the case of transplanted tumors, failed to cause any diminution in the amount of fibrous tissue production or to prevent the tumors from undergoing fibromatous changes. The effect of the hormone seemed, however, to be most pronounced on the glandular structures.

It was noted that in some of the adenofibromas the amounts of fibrous tissue present in the tumors far exceeded the amount of glandular tissue. The exact cause of this is not known at the present time although it is planned to correlate the histological character of the tumors with the age, past sex history and phase of sex cycle at the time of removal of the tumor. Heiman and Krehbiel [7] found that the age of the animal at the time of implantation with transplantable benign mammary tumors influenced the morphology of the growing implant. In immature animals soft transplanted fibroadenomas lost their glandular components and became hard, cellular, actively growing fibromas. In sexually mature females the glandular elements grew readily, sometimes becoming hyperplastic or developing into cysts. In old rats connective tissue elements again predominated but growth was slow and cells were few. Heiman and Krehbiel believed that the absence of the endogenous estrogenic hormone probably accounted for the scarcity of glandular structures in sexually immature rats and that the natural diminution in the amount of this substance explained the return to the fibrous type of growth in very old animals. In our rats, which seem to grow old prematurely, it may be that the diminished amount of endogenous estrogenic hormone associated with physiological old age may account for the preponderance of essentially fibrous mammary tumors.

In sections from some of the animals which had known tumors, as well as from 11 animals which had slight but discoverable focal thickenings of the breast, we were able to find minute areas of early but rather characteristic sclerosis. In rats which had proved newgrowths these foci had usually been undetected clinically as well as at autopsy and were found accidentally while studying microscopic sections of the mammary tissues. In the other animals they were in a stage where clinical recognition, or at least recognition while examining the mammary gland at the time of autopsy, was possible.

In these areas of focal thickening of the mammary tissue, areas which we believe are the initial stages in the development of an adenofibroma, the very earliest detectable change was a localized but rather characteristic increase in fibrous tissue. This local fibrous increase appeared first in somewhat different situations in the mammary gland in different animals.

In some animals the newly formed fibrous tissue developed in a rather diffuse manner but still in a localized portion of the mammary gland (Figs. 17 and 18). In such cases the initial proliferation of fibrous tissue occurred in the interlobular mammary fat and tended to involve a rather localized area in which a small number of mammary lobules was present. Sometimes this sclerosing focus could be palpated clinically but it was usually too small to be detected. As the connective tissue increased in amount it appeared gradually to surround and often to insinuate itself between the lobules in the immediate region (Fig. 18). Often the fibrosis seemed to begin in the tissues about a duct or ductule and to spread out along its course to surround lobules and even their acini. The latter structures would gradually become separated from each other by slowly proliferating connective tissue. Such proliferation was not usually attended by any great increase in the number of fibroblasts and the general appearance of the newly formed tissue was that of relatively normal but rather dense fibrous tissue. In some cases, however, there was evidence of active growth of the fibroblasts which then appeared more abundant than normal and were occasionally found in mitosis.

Before long, in the case of these initial tumorous lesions, a localized, circumscribed, slowly growing but typical adenofibroma which soon became encapsulated by compression of the adjacent normal tissue, was developed (Fig. 19). This focus then continued to grow slowly, becoming quite circumscribed in the breast and apparently always displaying, to some degree at least, the lobulations which characterize the normal mammary gland.

In other animals the initial stage in the development of the tumor was somewhat different from that already described although the change here, too, was characterized by a new proliferation of connective tissue. In these cases growth began not in the interlobular fat of the breast but rather in and immediately

around the secretory portions of a segment of the mammary gland. The connective tissue intimately associated with the glandular tissue in the lobules became slowly increased in amount until there were formed minute, almost microscopic nodules of sclerosis which developed separately in each lobule of the affected portion of the breast. The appearance of this part of the breast, seen with a hand lens, was not unlike a bunch of grapes, the major duct leading to the sclerosing region being the stem and the small fibrous nodules in the various lobules being the grapes. The histological appearance of this type of growth was that of numerous miliary adenofibromas arising close to each other, as in Figures 21 and 23, and being localized about a duct in one part of the breast.

This type of newgrowth thus arose in multiple foci in the delicate fibrous tissue which forms the lobular stroma. Collagen not only increased in amount but became dense, almost hyaline in character, although it rarely lost its fibrillar structure. There was little evidence of any considerable increase in the number of fibroblasts in the regions of proliferation and in no case were mitotic figures observed in the connective tissue cells. As growth continued in these multiple foci in a localized portion of the mammary gland, the individual nodules increased in size until in time, as shown in Figure 22, they came together and appeared to be compressed into a single mass which ultimately formed a solitary, well defined newgrowth, quite incapable of being differentiated from any of the large number of typical adenofibromas. The lobular character of such tumors seemed to be maintained and, although it has not of course been possible to follow the individual steps in the development of the bulky lobulated tumors of some of the rats, it is not unreasonable to assume that they arose in the manner here described.

It would appear, therefore, that in the minute, non-neoplastic appearing fibrous lesions which were found in the mammary glands of 11 rats without tumors, as well as in the breast tissue of a number of rats that had definite tumors elsewhere in the mammary gland, there were occurring the earliest stages of the development of the benign fibroepithelial tumor of the rat breast. We consider these changes to be in the nature of a pretumor fibrous hyperplasia which very soon acquires the characteristics of a typical benign newgrowth. But how the fibrous tissue is stimulated to

become increased in amount and density, why this increase should be localized in one breast or a small part of one breast, and why there are differences in the situation in which the fibrous tissue first begins to proliferate, we do not know.

There appeared to be still another manner, quite different from those already described, in which the development of some of these benign newgrowths might arise. Although they were few, some of the tumors were primarily adenomas rather than fibroepithelial neoplasms. The development of such tumors was less readily followed. Apparently the glandular hyperplasia which characterized this type of growth was a local intensification of the process which normally takes place when the glandular elements become more abundant under hormonal influence as, for example, at parturition. In the case of the adenoma, however, the change resulted in a permanent, though localized, increase in acinar tissue. In many places in these glandular tumors mitotic figures were found, indicating active growth, but since proliferative activity can be manifested in this way even in normal hyperplasia it is not possible to say that cells in mitosis are in any way characteristic of a tumor. The stimulus for the development of this predominantly glandular type of growth is not known.

There was suggestive evidence that, due to some influence not as yet understood, adenomas of the type just described could change into the fibroepithelial type previously discussed.

Summary and Conclusions

1. The gross and microscopic appearance of 149 mammary tumors which occurred spontaneously in the breasts of 141 female rats of the A–S strain has been described. The newgrowths consisted of 122 fibroepithelial tumors (adenofibromas or fibroadenomas), 10 fibromas, 11 adenomas, 5 cystadenomas and 1 carcinoma.

2. Atypical focal lesions in the breasts of 11 female rats without tumors and of a considerable number of rats with typical newgrowths were described in detail and the conclusion is drawn that they represent the earliest stages in the pathogenesis of the mammary neoplasms.

3. Preliminary bio-assays of tumor tissue for the presence of an estrogenic factor have not revealed detectable amounts of this

substance and it is our belief that, although the specific cause or causes for the growth of spontaneous mammary tumors in the rat breast are not known, hyperestrinism is not the sole etiological factor in their development.

4. A brief account of the effects of prolonged estrin administration on spontaneous benign mammary adenofibromas is given.

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DESCRIPTION OF PLATES

PLATE 136

FIG. 1. Rat A–S 5 with 2 tumors. The gross appearance of the larger tumors is well shown in this photograph.

FIG. 2. Exposed tumor of Rat A–S 2. Note the well defined capsule and the characteristic lobulations. A single large artery provided the chief blood supply.

FIG. 3. Low power view of a typical encapsulated fibroepithelial tumor from Rat A–S 3794. Note that the fibrous tissue increase is general, occurring within as well as between the lobules. × 20.

FIG. 4. Tumor from Rat A–S 3269. Fibrosis appears slightly less marked than in the tumor shown in Figure 3 and the glandular elements appear somewhat more abundant. × 10.

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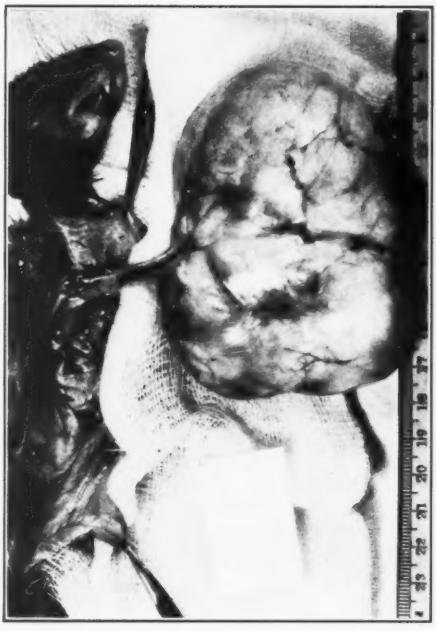

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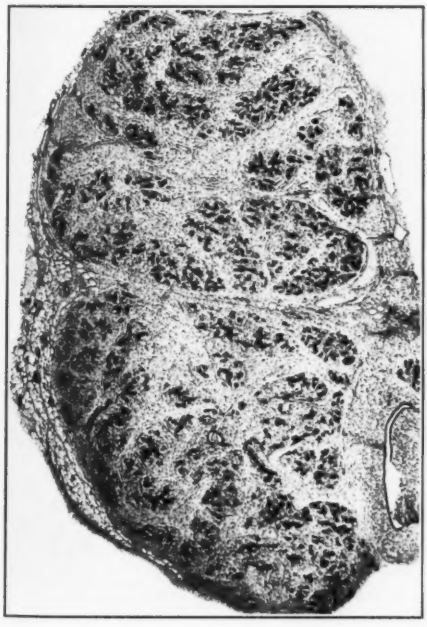

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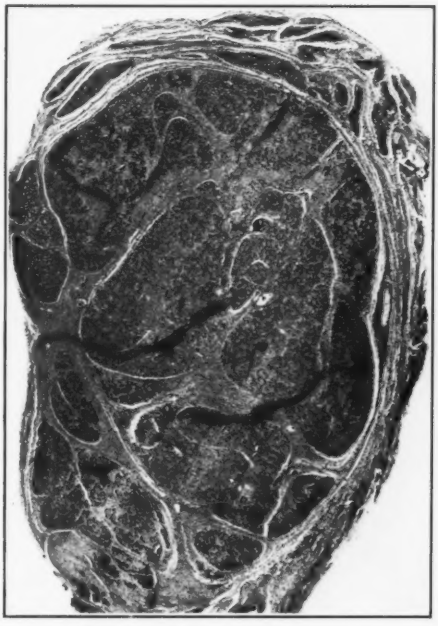

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Wright, Klinck and Wolfe

Mammary Tumors Occurring Spontaneously in Rats

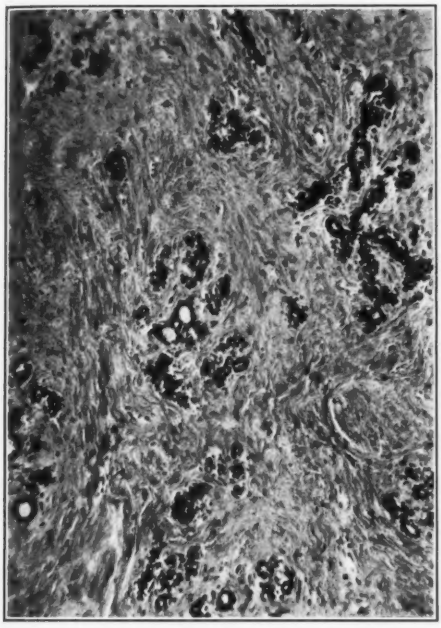
PLATE 137

FIG. 5. High power view of a typical fibroepithelial tumor showing the dense fibrous stroma and the sparsely scattered lobules composed of small ducts and their surrounding end buds or acini. Rat A–S 5. × 160.

FIG. 6. High power view of the edge of the tumor from Rat A–S 167 showing diffuse fibrosis with small but evenly scattered ductules without the lobular arrangement seen in Figure 5. Note the small, compressed extracapsular tumor which by accretion will soon become a part of the larger growth. × 160.

FIG. 7. Tumor from Rat A–S 2346. This rat was pregnant. Note the hyperplasia of the glandular tissue. × 10.

FIG. 8. High power view of a part of the tumor shown in Figure 7. Note the abundant secretion-filled acini. A milky fluid dripped from the cut surface of this tumor at the time of autopsy. × 160.



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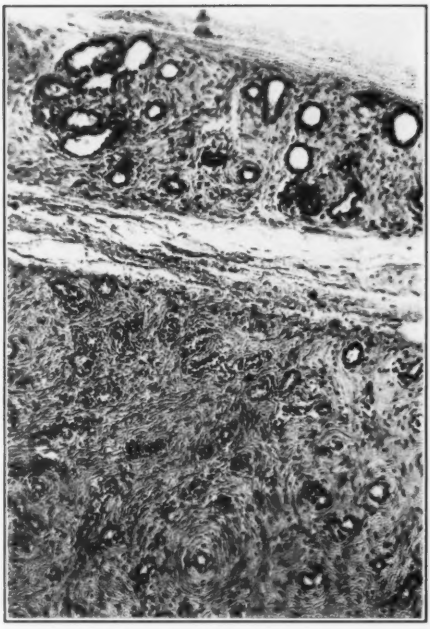

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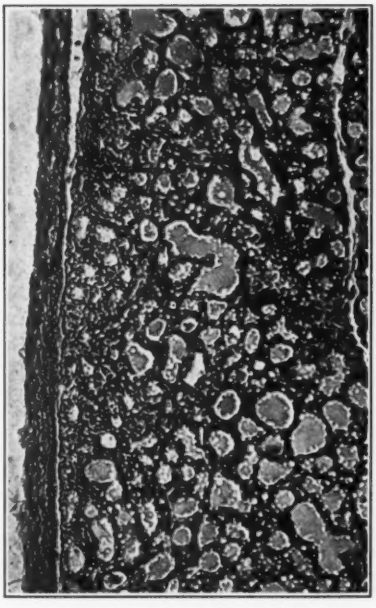

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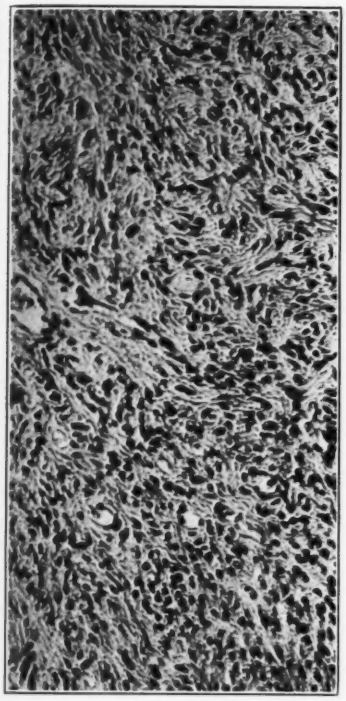
PLATE 138

FIG. 9. Low power view of a typical fibroma from Rat A–S 2. This tumor was of uniform histological structure throughout. The same newgrowth is shown in Figure 2. × 160.

FIG. 10. High power view of a fibroma from Rat A–S 2317 showing the character of the cells and the intercellular substance. One cell in mitosis is present. × 1500.

FIG. 11. A typical adenoma from Rat A–S 742. Glandular structures are abundant. × 20.

FIG. 12. A small, edematous, well encapsulated adenoma from Rat A–S 1974. × 30.



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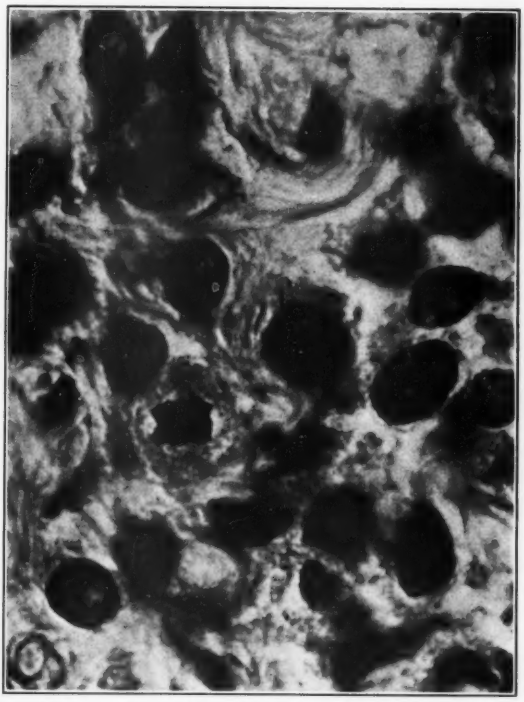

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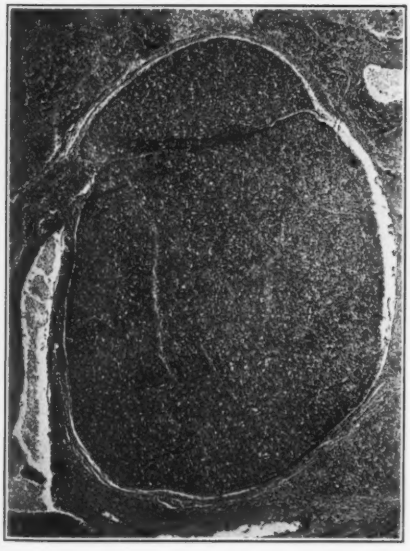

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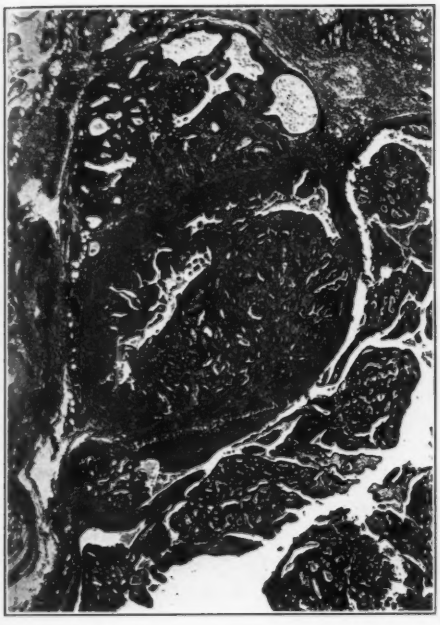
PLATE 139

FIG. 13. Low power view of a cystadenoma from the breast of Rat A–S 165. Note the disordered character of the growth. While this tumor grew rapidly and appeared grossly and histologically malignant, it gave rise to no metastases. Figures 15 and 16 show high power views of other portions of this tumor. × 25.

FIG. 14. A high power view of a portion of the atypical cystadenoma from the breast of Rat A–S 3830. This tumor, similar to that of Rat A–S 165, produced no metastases. × 200.

FIG. 15. High power view of a portion of the cystadenoma shown in Figure 13. Note the cellular papillary processes. × 200.

FIG. 16. High power view of a portion of the papillary process seen in Figure 15. Note the character of the growing epithelial cells. A single mitotic figure is present. × 1400.



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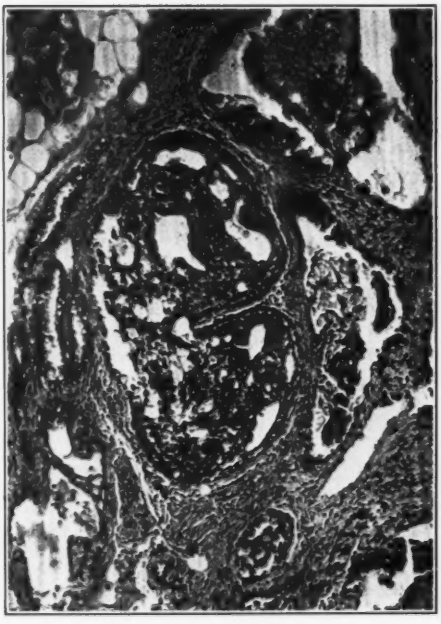

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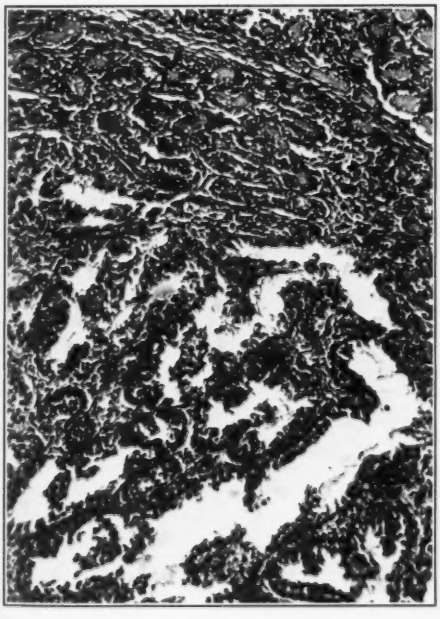

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PLATE 140

FIG. 17. Low power view of a very early stage of interlobular fibrosis in a single portion of the breast. This is considered to be the first step in the formation of a fibroepithelial tumor. Rat A–S 1160. × 10.

FIG. 18. A somewhat later stage of the diffuse pretumorous interlobular fibrosis which characterizes early tumor development. Rat A–S 262. × 10.

FIG. 19. An extremely small but typical fibroepithelial tumor from the breast of Rat A–S 670. The lobulated character of the growth is visible and beginning encapsulation can be seen. × 15.

FIG. 20. A small focus of pretumorous intralobular fibrosis in the breast of Rat A–S 493. Note how the fibrosis affects only separate lobules which are grouped about a few adjacent ducts. For a higher magnification of these foci see Figure 21. × 8.

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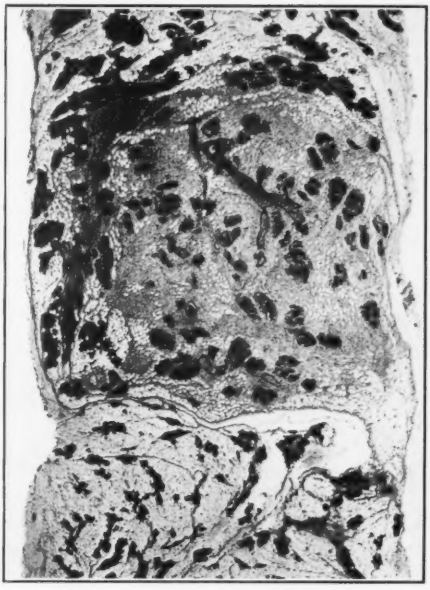

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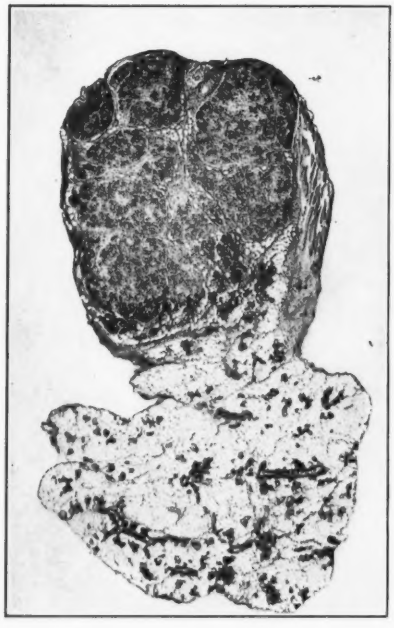

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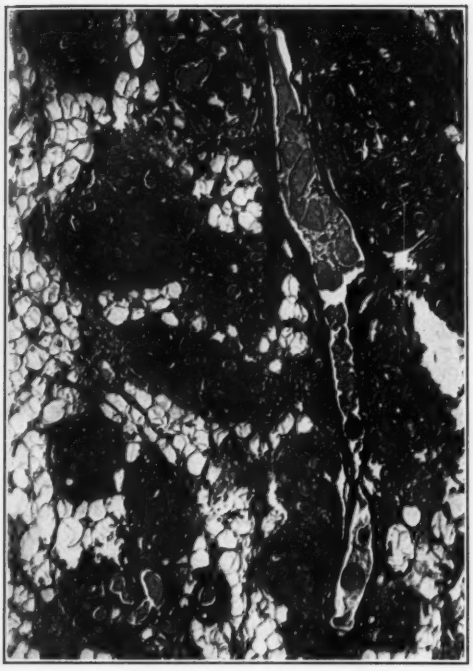
PLATE 141

FIG. 21. Higher power view of the intralobular fibrosis shown in Figure 20. Note the grape-like distribution of the focal lesions about the slightly distended duct. Rat A–S 493. × 80.

FIG. 22. A fibroepithelial tumor from Rat A–S 1078. This tumor undoubtedly arose in the same manner as that shown in Figures 20 and 21, but it is now a definite neoplasm. Note the manner in which the separate focal lesions (miliary adenofibromas) become compressed together to form a solid newgrowth. The lobulated character of the very large tumors could well be explained on the basis of this form of development. × 10.

FIG. 23. Higher power view of a portion of the tumor from Rat A–S 1078, shown in Figure 22. Note how the small fibrous lesions cluster in grape-like fashion about a small mammary duct. × 80.



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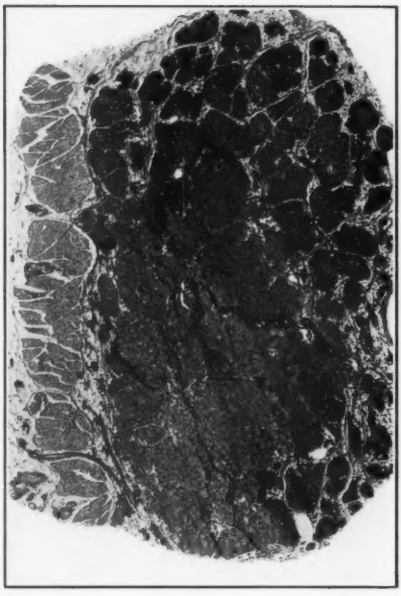

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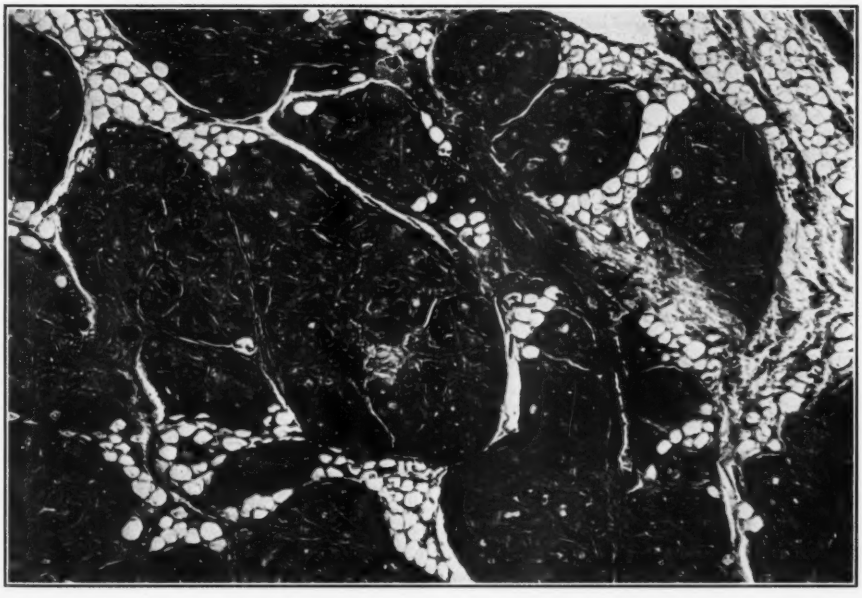

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Wright, Klinck and Wolfe Mammary Tumors Occurring Spontaneously in Rats

PRIMARY RETICULUM CELL SARCOMA OF THE SPINE *

Report of a Case with Autopsy

Jesse E. Edwards, M.D.

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Introduction

In 1939 primary reticulum cell sarcoma of bone was added to the list of primary bone tumors in a revised classification by the Registry of Bone Sarcoma of the American College of Surgeons.[1] The importance of the clinical recognition of this tumor lies in the fact that though malignant, it has a relatively good prognosis, as shown by the fact that in a series of 17 cases reported by Parker and Jackson,[2] 13 individuals were alive and apparently free from tumor 6 months to 14 years after the initial symptom. Seven of these were in good health for 10 years or longer.

In discussing the diagnostic features, these authors emphasized that the X-ray picture in primary reticulum cell sarcoma of bone is not specific and may be confused with that of osteomyelitis, Hodgkin's disease, Ewing's tumor and osteogenic sarcoma. It cannot, therefore, be used as an absolute criterion in establishing the diagnosis. The microscopic appearance of the tumor is, however, characteristic, and the diagnosis must rest on this alone.

Parker and Jackson concluded that in each of their cases the tumor originated in bone, and constituted an entity different from reticulum cell sarcoma of lymph nodes. They found that primary reticulum cell sarcoma of bone appears largely in adolescents and in young adults, 13, or 77 per cent, of their cases occurring under 40 years of age. On the other hand, reticulum cell sarcoma of lymph nodes is usually seen in middle life or in old age, 85 per cent of all cases being found in individuals over 40 years of age. Chart 1 illustrates the age distribution in 19 cases of primary reticulum cell sarcoma of bone and in 102 cases of lymph node reticulum cell sarcoma, all of which were proved by microscopic examination of tumor tissue.

* Read in part before the Third International Cancer Congress, Atlantic City, N. J., September 11, 1939.

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The anatomical distribution of primary reticulum cell sarcoma of bone frequently varies from that of metastatic lesions derived from primary reticulum cell sarcoma of lymph nodes, for while the spine and the calvarium are the most common sites of involvement in metastatic reticulum cell sarcoma, these bones were not involved in any of the cases of primary bone tumor reported by

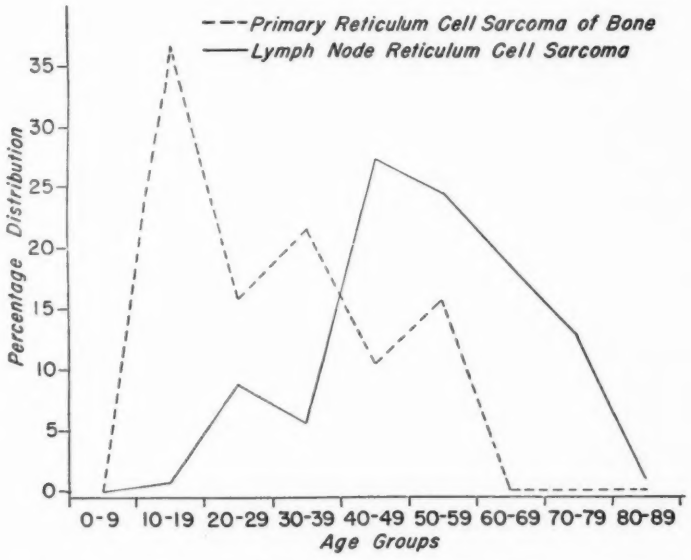

CHART 1. Graphic representation of age distribution in 19 cases of primary reticulum cell sarcoma of bone and 202 cases of reticulum cell sarcoma of lymph nodes.

Parker and Jackson. The distribution in their cases was as follows: femur 5 cases, clavicle 4 cases, tibia 3 cases, humerus 3 cases, and mandible and scapula, 1 case each.

Moreover, indicating that these authors were dealing with a distinct entity and a primary bone tumor is the fact that with amputation or resection patients in their series have remained alive as long as 14 years after the onset of the initial symptom.

Although reticulum cell sarcoma frequently arises in lymphoid tissue, it does not always do so. The cases reported by Parker and Jackson, as well as the case reported here, are examples of reticu-

lum cell sarcoma arising elsewhere than in lymphoid tissue. Yuile [3] reported a primary reticulum cell sarcoma of the brain, and Lofdahl and Levine [4] observed a case in which the tumor was considered to be primary in the chest wall.

In this paper a case which showed a primary reticulum cell sarcoma of the spine and which came to autopsy is reported. So far as known, this is the first report of an autopsied case of this tumor.

Report of Case

Clinical History: C. H. (B.C.H. No. 893,252), a 59 year old, white married woman, was admitted to the Boston City Hospital on March 27, 1938, because of paralysis and loss of sensation in both legs.

In April, 1929, during a course of antiluetic therapy with neoarsphenamine, she developed jaundice, anemia, leukopenia and thrombocytopenia. She recovered gradually but continued to have a moderate anemia and leukopenia. Following this episode she was treated with bismuth and the blood Wassermann reaction ultimately became negative.

In July, 1934, she was admitted to the hospital because of indefinite abdominal pain. On this admission the spleen was described as greatly enlarged. The blood counts still showed a moderate anemia and leukopenia. Splenectomy was advised but the patient refused this procedure.

In the summer of 1935 she complained of pain in the lower back. This lasted but a short time, however, and she had no further pain until 2 years later, 15 months before her death. At this time she experienced pain in the lower back on motion, such as rolling over in bed or bending. The pain which at first radiated to the right flank increased steadily in severity and ultimately radiated to the hip and to the legs. Shortly before admission to the hospital she developed complete paralysis of both legs associated with loss of sensation in the legs and thighs.

Physical Examination: On physical examination the heart and lungs were essentially normal. The spleen was firm and markedly enlarged, filling the whole left side of the abdomen. On percussion the lower margin of the liver was demonstrated 4 cm. below the costal margin. Tenderness was present in the right upper quadrant. There was marked tenderness in each costovertebral angle. The lower extremities showed flaccid paralysis and absent knee and ankle jerks. Anesthesia was present in the legs to the hips anteriorly, and posteriorly there was absence of sensation in the legs and so-called saddle distribution of anesthesia. She was incontinent also.

Laboratory Studies: Blood studies revealed a moderate anemia, the hemoglobin varying from 69 per cent on admission to 52 per cent at the time of death. The red blood cell count on admission was 3,690,000 per cmm., falling to 2,340,000 shortly before death. The total leukocyte count fluctuated between 5250 and 1600 cmm. The differential counts were essentially normal. The urine showed a trace of albumin and many white blood cells. Cultures of the urine revealed *B. coli* and an alpha hemolytic streptococcus.

X-ray studies of the spine showed a slight narrowing and rarefaction of the bodies of the fifth and sixth thoracic vertebrae. The body of the first lumbar

vertebra showed irregular mottling and slight narrowing. There was no evidence of bone formation. The long bones and the pelvis showed no evidence of tumor.

Course of Illness: The lower extremities remained paralyzed and the severe pain in the back continued throughout the period of hospitalization.

During the 8th, 9th and 10th hospital weeks, fifteen X-ray treatments were given to the right dorsal spine through two portals totalling 6000 r units. Under this therapy the spleen shrank to about two-thirds its former size and the pain in the legs diminished in intensity. X-ray therapy was discontinued because of the increasing size of a sacral decubitus ulcer. The temperature was essentially normal until 4 days before death, at which time it gradually became elevated, reaching 105 F. terminally.

Death occurred at the end of the 12th hospital week (June 20, 1938). The final clinical diagnoses were Hodgkin's disease of the spleen with metastases to the spine and involving the cord and cauda equina, bronchopneumonia and latent syphilis.

Postmortem Examination *

The gross anatomical diagnoses were: malignant tumor of undetermined nature, involving the twelfth thoracic and the upper two lumbar vertebrae, the spinal epidural space and the retroperitoneal tissues; metastases to the para-aortic and the iliac lymph nodes; biliary cirrhosis of the liver; chronic cholecystitis with cholelithiasis; dilatation of the cystic and the common bile ducts; splenomegaly; acute cystitis; bronchopneumonia involving the right middle lobe; pulmonary congestion and edema; and pelvic peritoneal endometriosis.

The body was that of a well developed and well nourished adult white woman. An ulcer measuring 2.5 cm. in diameter and 0.6 cm. in depth was present on the lateral aspect of the lower third of the right leg and was consistent with a varicose ulcer. Sloughing voluntary muscle was seen at the base of the ulcer. No superficial lymph nodes were palpable.

The peritoneal cavity contained about 200 cc. of clear, straw-colored fluid. The mesenteric lymph nodes were not enlarged. The posterior cul-de-sac of the pelvic peritoneum was obliterated by fibrous adhesions. Among these adhesions, as well as within the serosa of the uterus, anteriorly and posteriorly, were a few small cysts measuring up to 0.1 cm. in diameter, all containing fluid; in some cysts the fluid was clear and colorless, while in others it was yellowish brown.

The pleural surfaces were smooth. There were no adhesions or

* Autopsy No. A 38–340.

free fluid in either cavity. The surfaces of the pericardial cavity were smooth and glistening.

The body of the first lumbar vertebra was somewhat compressed and largely replaced by firm, pale grayish tumor tissue containing spicules of bone, and appeared to be the focus from which the tumor extended upward and downward to invade the adjacent halves of the body of the twelfth thoracic vertebra and the second lumbar vertebra, posteriorly to invade the epidural space and laterally to involve the belly of each psoas major muscle. In its lateral course the tumor was somewhat more extensive on the right, not only involving muscle but also displacing the right adrenal anteriorly and invading it posteriorly. The bodies of the third, fourth and fifth lumbar, and ninth, tenth and eleventh thoracic vertebrae showed the usual architecture and the marrow was bright red in color. No tumor was present in these vertebral bodies. Above the level of the ninth thoracic segment the spine was not sectioned. However, careful palpation of the spine failed to reveal any deformity of the upper thoracic region. In the lower one-third of the spinal canal the extradural space was found to contain tumor tissue continuous with and similar to that present in the first lumbar vertebra. The extradural tumor measured up to 0.4 cm. in thickness and appeared to invade the dura from without inward. The pia-arachnoid was not remarkable.

The para-aortic and iliac lymph nodes were slightly enlarged, measuring up to 2 cm. in diameter. The parenchyma of the lymph nodes was replaced by tissue similar to that of the tumor mentioned above. These were the only metastases found. There were no metastases present in any of the viscera.

The heart weighed 240 gm. and was essentially negative.

Bronchopneumonia was present in the middle lobe of the right lung. Congestion and edema were the only abnormalities in the remaining pulmonary lobes. The tracheobronchial lymph nodes were small and anthracotic.

The spleen was greatly enlarged, weighing 1180 gm., and presented a tense, slate colored capsule. The pulp was brownish red and firm, apparently containing an abundance of fibrous tissue. No tumor nodules were observed. The splenic vein was widely dilated.

The gastro-intestinal tract showed no abnormalities.

The liver was of usual size, weighing 1660 gm. The capsule was slightly granular and reddish brown. On cut section the portal areas appeared somewhat enlarged. A faintly gritty sensation was elicited on scraping the cut surface. There was no demonstrable tumor tissue.

The gall-bladder and all the extrahepatic biliary ducts were moderately dilated. The lumen of the gall-bladder contained moderate numbers of soft, dark brown amorphous flakes.

The pancreas was essentially normal. The surrounding lymph nodes were soft, pink and moderately prominent, the largest measuring 1.5 by 1 cm.

The combined weight of the kidneys was 300 gm. They were of the usual weight and size and showed no evident gross abnormalities. The pelves were normal. Each ureter showed an occasional focus of slight dilatation.

Posteriorly the right adrenal was partly replaced by the invading retroperitoneal tumor mentioned above. The left adrenal was of the usual appearance.

The bladder showed evidence of a mild cystitis.

Microscopic Examination

The histological appearance of the tumor is that of a reticulum cell sarcoma (Fig. 1). The stroma is loose and moderately vascular. The tumor cells are distributed evenly and in many instances are separated from one another by delicate strands of collagen.

The nuclei are all moderately large and vary from one and a half to three times the size of a lymphocytic nucleus. Some are round, others oval, and many are kidney shaped. Nuclear chromatin is sparse and is present in the form of a delicate, rather evenly distributed network. In many instances there is a prominent central nucleolus. Mitotic figures are present in small numbers. An occasional binucleate cell is seen but no Reed-Sternberg cells are found. The cytoplasm of the tumor cells which is either acidophilic or basophilic is fairly abundant and stains faintly.

Evidence of ameboid activity on the part of the tumor cells is seen in their variations in shape. Some are roughly spheroid, some are narrow and elongated, while still others show cytoplasmic and nuclear pseudopod-like processes.

The distribution of the reticulum is characteristic of this type

of tumor in that it is found surrounding individual cells as well as groups of tumor cells (Fig. 2). The scharlach R stain reveals fat droplets in the cytoplasm of scattered tumor cells.

A section of a para-aortic lumbar lymph node shows its structure to be replaced by tumor tissue similar to that seen in the primary tumor.

A representative peripancreatic lymph node shows no tumor. A few, hemosiderin-laden macrophages are present in the lymph sinuses and in the connective tissue. Section of a tracheobronchial lymph node also shows no tumor.

The spleen shows sclerosis and hemosiderosis of the red pulp. In several areas there are fibrous nodules rich in elastic tissue and in hemosiderin. No tumor is present.

The liver shows the usual hepatic architecture with no apparent increase in connective tissue. The portal areas show minimal infiltration with lymphocytes and macrophages, some of the latter containing black granules. A moderate number of Kupffer cells contain hemosiderin.

The remaining organs show no tumor and have no direct bearing on the oncological aspect of this case. The kidney shows an early acute pyelonephritis. Bronchopneumonia is present in the lung.

The marrow of a vertebral body not involved by tumor shows a moderate hyperplasia of the hematopoietic cells with fat cells present in moderate numbers. A few lymph follicles are present. There is a moderate amount of phagocytosis of erythrocytes. Macrophages in moderate numbers are laden with golden yellow pigment.

Discussion

The body of the first lumbar vertebra replaced by tumor appears to be the central point from which there was direct extension to the soft tissues on each side of the spine, to the lower half of the adjacent twelfth thoracic vertebra, to the upper half of the second lumbar vertebra, and posteriorly to the spinal epidural space. The distribution of the involved lymph nodes is consistent with the anticipated sites of local lymph node metastases derived from the area involved by the primary tumor mass.

The splenomegaly and evidence of involvement of bone led to

the erroneous clinical diagnosis of Hodgkin's disease of the spleen with secondary involvement of bone. At autopsy there was definite enlargement of the spleen but no gross or microscopic evidence of Hodgkin's disease or of any tumor. The splenic enlargement had no apparent relation to the tumor of the spine. The spleen showed fibrosis of the pulp, hemosiderosis, and nodules of fibrous tissue rich in deposits of hemosiderin. This picture is sometimes seen in obstruction to the venous outflow of the spleen. In this case at the time of autopsy the splenic enlargement was attributed to a supposed cirrhosis of the liver, but in the absence of histological confirmation of the gross diagnosis of cirrhosis of the liver one hesitates to consider the liver as contributing to the obstruction of the splenic venous circulation.

The presence of metastases in local lymph nodes is consistent with the observations of Parker and Jackson. In their original study of primary reticulum cell sarcoma of bone they indicated that metastases in this disease may not occur until late, even in the presence of a massive primary tumor. Ordinarily when metastases occur the local lymph nodes are involved first. Later blood stream spread to other bones and the lungs may ensue.

In the case reported here the earliest symptom was directly referable to the back and appeared over 3 years before death, yet metastases were demonstrated only in local lymph nodes. These were rather small even though the primary tumor had grown to a bulky size.

The presenting symptom of pain referable to the site of the tumor in this case is consistent with the observations reported by Parker and Jackson.[2] In their series of 17 cases, 13 individuals presented themselves for study because of this symptom. These authors indicated that the pain may be severe and is not relieved by rest. They emphasized that this symptom is not specific for primary reticulum cell sarcoma of bone for it occurs in bone tumors of other types.

Paralysis and loss of sensation in the lower extremities and incontinence are explained on the basis of compression of the lower spinal cord and cauda equina by the tumor which had extended into the epidural space. Absence of fever, except as associated with the terminal pneumonia and pyelonephritis, is entirely consistent with the report of Parker and Jackson who state that fever was not present in their cases.

The presence of anemia and leukopenia appears in this case to have preceded the tumor of the spine. These alterations in the peripheral blood were first noticed in April, 1929, 9 years before the death of the patient, and may well have been due to the antiluetic therapy. In the 17 cases reviewed by Parker and Jackson there was no abnormality in the peripheral blood.

It has been emphasized that the X-ray picture in primary reticulum cell sarcoma of bone is not pathognomonic and that on X-ray examination this tumor has been confused with other lesions of bone. Parker and Jackson state that the lesion is largely osteolytic but that new bone formation does occur, usually in association with pathological fracture. The case here reported conforms to these observations in that the X-ray picture was largely osteolytic.

Palpation at the time of autopsy failed to reveal any abnormality in the outline of the fifth and sixth dorsal vertebrae. No sections of these bodies were, however, taken. If the lesions in the fifth and sixth dorsal vertebrae were due to reticulum cell sarcoma, one would of necessity have to postulate either that the tumor arose at these levels or that the lesions were metastases from the tumor primary in the first lumbar vertebral body. The latter supposition would seem to be logical on the basis that the lesion in the lumbar region was the site of the initial symptom of pain in the back, that the lumbar tumor was by far more extensive grossly, and that lymph node metastases were confined to the lumbar and iliac regions.

Summary

A case of a 59 year old woman with primary reticulum cell sarcoma of the spine is presented. Symptoms referable to the primary tumor were present at least 3 years prior to death. The bone tumor was extensive, yet metastases were small.

The X-ray picture was not characteristic during life and was thought to represent a metastatic lesion.

Pain not relieved by rest was the outstanding symptom, as has previously been noted to be the case in such tumors.

Note: I am indebted to Dr. Leslie S. Jolliffe for the microphotographs and to Miss Evelyn M. Glidden for preparation of the chart.

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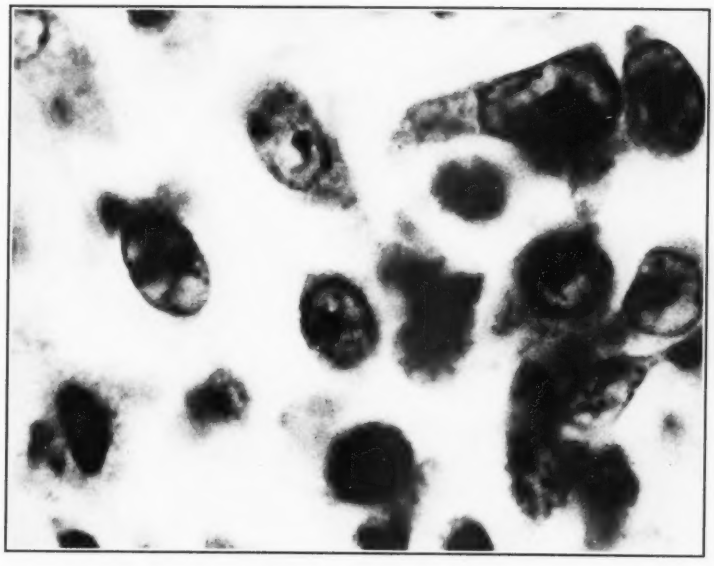
DESCRIPTION OF PLATE

PLATE 142

FIG. 1. Microphotograph of primary tumor showing irregularity in the shape of the nuclei, ameboid cytoplasmic processes in the cell at the upper level of the field, and a mitotic figure in the left lower corner. Phloxine-methylene blue stain. × 1000.

FIG. 2. Microphotograph of primary tumor showing reticulum encircling single cells. Foot's modification of Bielschowsky's stain for reticulum. × 1000.



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Edwards Primary Reticulum Cell Sarcoma of Spine

COMPARATIVE EXPERIMENTAL STUDIES OF 200 KILOVOLT AND 1000 KILOVOLT ROENTGEN RAYS *

II. The Biological Effects on the Bone Marrow of the Albino Rat

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Roentgen therapy by means of supervoltage rays, having reached the stage of widespread clinical application, has precipitated the need for the appreciation of actual histological effects produced by this type of irradiation. Unfortunately controlled data have not been recorded so that contrast with the biological results produced by ordinary high voltage rays is not possible without further investigation. For this reason a series of experiments has been performed in an attempt to establish first the histological lesions produced in normal mammalian tissues by 200 kv. roentgen rays, and secondly to contrast with these lesions those caused by supervoltage (1000 kv.) rays. In a previous study [1] the effects of equal quantities of roentgens of each of these rays on the epiphyses of young albino rats were described. It is our purpose in this presentation to record a description of the changes produced in the bone marrow of the same animals under similar conditions.

Methods

Since the experimental plan and animals are identical with those previously reported,[1] only a brief résumé of the methods utilized will be outlined. The series studied consisted of 95 albino rats roughly evenly divided as to sex. They were all 50 to 54 days old at the time of irradiation. The diet consisted of the Sherman BMS regimen recommended by Russell.[2] The animals were housed in open meshed wire cages and, except for an occasional transient period of anorexia immediately following irradiation, all the rats gained weight steadily. Only 2 succumbed spontaneously.

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The animals were divided into two major groups, one of which (Group I) was subjected to high voltage (200 kv.), and the other (Group II) to supervoltage (1000 kv.) roentgen rays. Each of the major groups was further divided into six smaller subgroups. One of these (Subgroup A) received no treatment and was used as a control. The remainder received a single treatment each of 600 r (Subgroup B), 1200 r (Subgroup C), 1800 r (Subgroup D), 2400 r (Subgroup E), and 3000 r (Subgroup F) over the lateral aspect of the right thigh. The animals were sacrificed by decapitation at staggered intervals of from 1 to 19 weeks after irradiation.

The qualities of the two roentgen machines used were as follows:

Group I Picker-Waite Machine		Group II Van der Graaf Generator
200,000 volts	Constant potential	1,000,000 volts
20 cm.	Distance	50 cm.
20 milliamp.	Current	0.85 milliamp.
0.25 mm. copper, 1.0 mm. aluminum	Filtration	3.0 mm. lead, 8.0 mm. copper
0.75 mm.	Copper half value layer	10.5 mm.
300 r/min.	Intensity	133 r/min.
10 cm.	Cone	10 cm.

Dosages are expressed in roentgens as measured in air with the Victoreen dosimeter.

The animals were immobilized on a plywood board and total treatment was given at a single sitting. Immediately following sacrifice the right femur was disarticulated and split longitudinally between the condyles. With a blunt probe a pencil of marrow was removed from the exposed shaft cavity, placed on a piece of filter paper and fixed in Zenker's fluid plus 5 per cent acetic acid. Specimens were sectioned in paraffin and stained with phloxine-methylene blue.

Observations

In order to facilitate description, the appearance of the marrow of untreated controls will be initially outlined. The changes produced by 200 kv. irradiation will then be recorded, and finally such observations as were made following the use of supervoltage rays will be appended. The time intervals referred to represent

the day or week following the application of irradiation. A chart denoting the experimental schedule has been included in the first report of this study [1] and will not be reproduced in this publication.

Normal Marrow

The femoral bone marrow in all of the control rats in this series was intensely cellular and contained very little fat (Fig. 1). Component elements were widely intermixed but were preponderantly erythrogenic, normoblasts constituting approximately one-half of the total number of nucleated cells. One-quarter to one-third of the cells were mature (segmented and non-segmented) granulocytes of which neutrophils were considerably more numerous than eosinophils. The remainder of the cells were composed about equally of myelocytes and undifferentiated stem cells. Megakaryocytes were much more abundant than in human marrow, numbering three to six per high power field. The degree of cellularity and proportionate numbers of constituents were quite constant among all of the controls.

200 Kilovolt Roentgen Rays

The hematopoietic pattern was affected by all dosages used in these experiments. Changes were minimal in degree in those extremities receiving 600 r, were moderately severe with 1200 r, and were maximal with doses of 1800 r or more.

Between the 5th and 14th day after irradiation a depression of total cellularity to almost one-half that of normal occurred in each instance (Fig. 2). Associated with this reduction of blood cells, fat spaces increased in number and prominence but there were no evidences of intrinsic cellular degeneration noted. This primary diminution of cellularity was almost wholly due to marked reduction in the number of normoblasts. Other marrow elements (neutrophilic and eosinophilic leukocytes and the immature cell group composed of myelocytes and stem cells) were less obviously affected in this phase. However, following the lower dosages a barely significant rise in primitive cells was noted. Wünsche [3] also observed an early reduction of normoblasts following irradiation of marrow, and Clarkson, Mayneord and Parsons [4] stated that the erythrogenic apparatus of the rat was unusually radiosensitive.

During the succeeding 2 week period a rapid return of the marrow to normal numerical content was observed (Fig. 3). Piney,[5] Wünsche,[3] and Casati [6] have all recorded evidence of prompt marrow recovery subsequent to the initial depressant effects of irradiation. During the period of apparent recovery our animals showed displacement of marrow fat by cellular replenishment and relatively few and small fat vacuoles were observed. Qualitative analysis at this stage showed a variation in the cellular pattern roughly related to the amount of irradiation applied. Marrow subjected to the lower dose range (600–1200 r) showed an absolute increase in all types of hematopoietic elements which, however, in only a few cases returned to pretreatment levels. On the other hand, with the larger doses the increase in cellularity was contributed to almost entirely by polymorphonuclear granulocytes, the less mature elements and nucleated red cells exhibiting little evidence of recovery. Wünsche has observed the appearance of a "right shift" with increased maturity of leukocytes in the peripheral blood following irradiation which probably was the result of a similar medullary polynucleosis. Transient leukocytosis subsequent to roentgen therapy has been noted by Wright and Bulman,[7] and by Aubertin and Beaujard.[8]

The secondary rise in marrow elements persisted for only a brief period and after the 35th day all treated marrows showed a progressive lessening of cellularity varying in rapidity and degree directly with the intensity of the initial treatment. In those subgroups which had received 1800 r or less the point of maximum decrease was attained during the 5th to 9th week. However, the degree of hypocellularity reached in these rats was rarely less than about one-half that of the normal marrow. Retention of marrow bulk was achieved by reexpansion of fat without recognizable stigmata of degeneration among hematopoietic elements. In the subgroups to which 2400 r and 3000 r had been administered a condition verging on aplasia was produced. This was readily apparent at about the 7th week after irradiation (Fig. 4). The depletion of cellular marrow was in many instances associated only with a compensatory increase of fat. In a few, particularly in the epiphyseal and metaphyseal regions, there appeared a somewhat edematous, granular fibrous tissue occasionally containing foci of mucinous degeneration (Fig. 5). The few marrow cells which

persisted were mature neutrophilic and eosinophilic granulocytes. Occasional mononuclear phagocytes with either cytoplasmic vacuolization or ingested hemosiderin were seen. Lesions of this character, both those with partial and those with complete cellular depletion, once established apparently persisted throughout the period of the experiments without evidence of return to normal.

Megakaryocytes regularly exhibited degenerative phenomena with all doses, the degree of which was proportionate to the amount of therapy. In general there was progressive diminution in number and a relative increase of older and effete forms. Such senility was evidenced by increased cytoplasmic eosinophilia and the appearance of shrunken pyknotic nuclei.

In résumé, the initial effects of irradiation were reflected by a sharp diminution of normoblasts. In Groups B and C (600 and 1200 r) this was mitigated by a transient reappearance of these elements between the 21st and 35th days. Animals subjected to higher doses exhibited only a minor secondary increase of normoblasts although a somewhat more marked simultaneous increment of mature granulocytes was observed. After the 5th week normoblasts were reduced to a minimum fairly rapidly.

In all subgroups during the first 2 weeks succeeding treatment, although stem cells and myelocytes contributed very slightly to the marrow content, they manifested a moderate increase in relative and absolute proportions. This phenomenon which was much less evident with higher doses attained and barely held its peak from the 14th to the 28th day. At the end of this brief phase there occurred a regression of these cells to minimal levels.

Mature granulocytes were present only in small numbers in the femoral marrow of untreated control rats. This was contrary to the observations of Stasney and Higgins [9] whose data, however, were obtained from a somewhat older age group. Following irradiation with all doses utilized a comparatively rapid increase in polymorphonuclear granulocytes was noted. These cells reached a maximum variously between the 1st and 4th week. At the end of this period there was prompt retrogression in absolute numbers so that by the 35th to the 49th day a subnormal level was reached. It is noteworthy, however, that the curve of polymorphonuclear regression lagged behind that of less mature elements so that a relative preponderance of mature granulocytes with a reversal of

the normal predominance of myelocytes over polymorphonuclears was attained and persisted throughout the course of the study (Chart 1). As a result, at the time the late effects of irradiation supervened, although hypocellularity was preeminent, neutrophils and eosinophils were more abundant than any of the other cells.

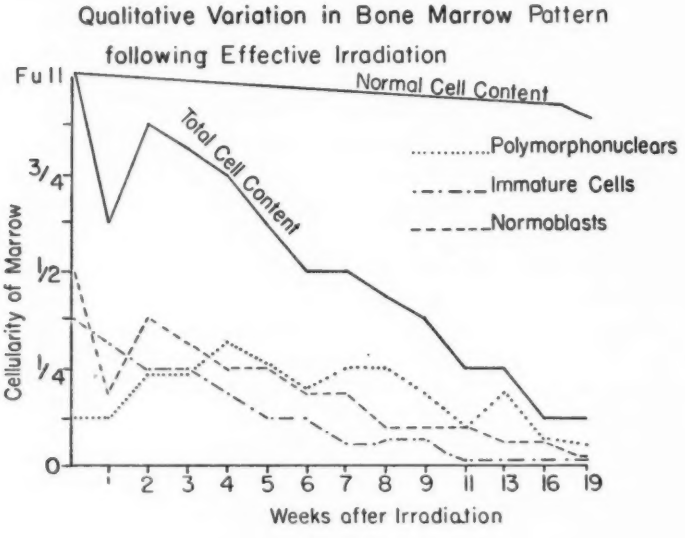

CHART 1

1000 KILOVOLT ROENTGEN RAYS

Comparative studies of the changes effected in the marrow of animals receiving supervoltage irradiation demonstrated reasonably conclusively that there was no significant or constant difference in the lesions produced by either form of roentgen rays (Chart 2). Similar amounts of roentgens as measured with the Victoreen dosimeter produced essentially similar marrow modifications regardless of whether the source of the irradiation was the 200 kv. or the 10000 kv. apparatus. This is in accordance with the observations previously recorded in which it was observed that similar results were produced by the two types of irradiation upon the growing epiphysis of the rat.[1] The slightly delayed onset of cellular changes following 1000 kv. radiation noted in the epiphyseal studies was not evident in the bone marrow. As shown

by Chart 2, the extent and chronological appearance of the lesions was the same in both groups.

Discussion

Isaacs has stated that the fundamental effect of X-rays on hematopoietic tissues is one of acceleration of physiological activity.[10, 11] Primitive cells are said to proliferate more rapidly and

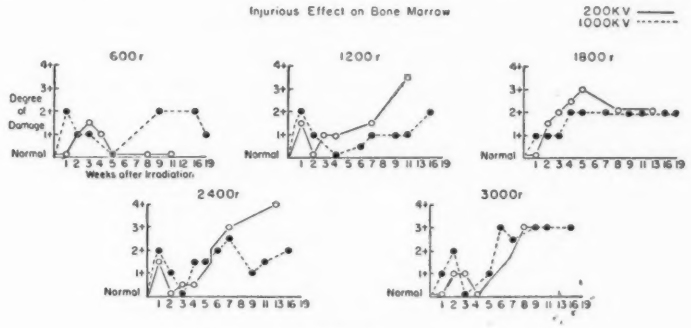

Chart 2

cells of an intermediate grade of maturity to proceed toward ultimate maturation in a shorter span of time than would normally be the case. Wünsche has concurred in this belief.[3] Although there can be little question that the end result of irradiation in the blood-forming system is destructive,[6, 12, 13] there is some evidence in our observations to confirm the presumption that earlier in the course of events a stimulus toward rapid maturation is apparent.

The initial regression of normoblasts in all treated rats may be considered to represent the result of any one of several factors. It is probably not representative of an immediate destructive influence since none of the stigmata of such a process were observed (nuclear fragmentation, necrosis, hemosiderosis, and so on). Another alternative, the extrusion of nucleated red cells into the peripheral circulation, is unlikely. This phenomenon has been observed only on rare occasions. Unfortunately peripheral blood studies were not performed on our subjects. A more reasonable thesis is contingent on a rapid maturation of preexisting normoblasts into erythrocytes. Wright and Bulman,[7] and Minot and

Spurling [14] have recorded instances of immediate increases of peripheral red cells following irradiation. Isaacs [15] has observed a rise in reticulocytes and young "granule" erythrocytes in the circulating blood under similar circumstances.

Additional evidence of accelerated maturation has been provided by the appearance of a secondary rise in normoblasts only in those subgroups (low dosages) in which there had been a slight but significant increase in the number of primitive cells concomitant with the primary diminution of normoblasts. During the 2nd to 4th weeks, the period in which the transitory secondary rise of normoblasts occurred, the number of primitive elements having reached a peak began simultaneously to diminish. In a parallel fashion there was an early and progressive increase in the number of mature granulocytes continuing well beyond the period of diminution of myelocytes. The preponderance of polymorphonuclears over myelocytes, a relationship not evident in the untreated controls, indicated accelerated maturation in the granulocytic as in the erythrogenic series.

It is reasonable to assume, therefore, that within 1 to 2 weeks following stimulation by irradiation, normoblasts probably matured and became erythrocytes. There was consequently a fall in the number of these elements in the marrow. Concomitantly, with myelocytes following a similar trend, there was a progressive rise in the number of marrow polymorphonuclears. A secondary or "regenerative" return to normal numerical cellular content occurred between the 2nd and 4th weeks. This presumably resulted from the enhanced maturative and proliferative activities of the preexisting primitive cells. Subsequent to this phase, exhaustion or inhibition of stem cells supervening, a progressive atrophy of the marrow associated with a permanent "shift to the right" developed.

Chart 1 represents an attempt to demonstrate graphically a composite picture of the effect of irradiation on the bone marrow pattern. The progressive depletion of marrow content interrupted briefly at the 2nd week may be readily appreciated. There is progressive diminution in primitive cells and myelocytes, a transient increase in normoblasts, and a steady rise in polymorphonuclears. The small secondary peak in primitive elements following small amounts of roentgen rays is not shown.

Summary

The application of graded doses of irradiation to the bone marrow of the albino rat produces a prompt diminution in cellular content proportionate in amount to the dose. This is initially contributed to almost wholly by normoblasts and is invariably followed by a short period of apparent regeneration which subsequently gives place to a progressive decline in cellularity. The degree of hypocellularity attained is directly proportionate to the dose administered. Qualitatively there is ultimately a depression of normoblasts, primitive cells, and myelocytes with a persistent preponderance of mature granulocytes.

Comparative analyses after irradiation with both 200 kv. and 1000 kv. roentgen rays demonstrate no essential difference in the effects on the marrow of equal amounts of roentgens of both types of rays.

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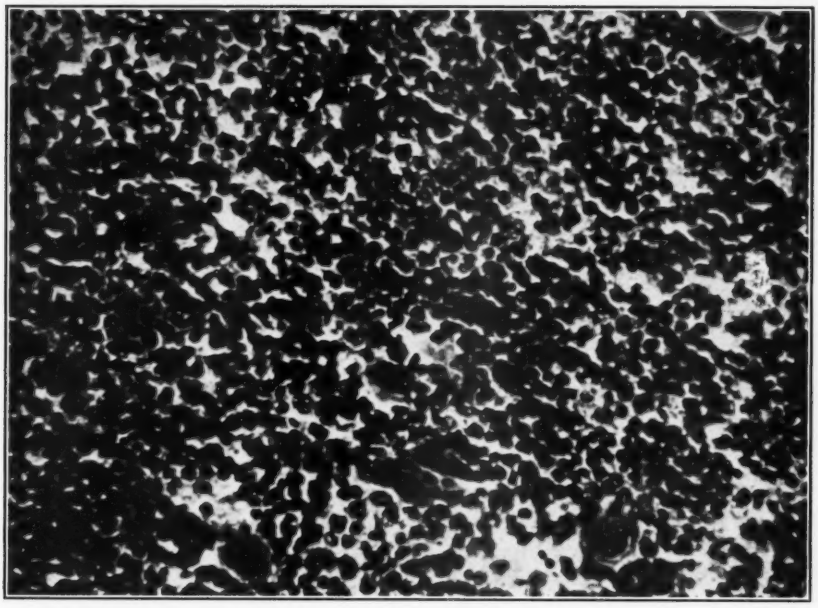
DESCRIPTION OF PLATES

PLATE 143

FIG. 1. Marrow from the shaft of the femur of an untreated 102 day old albino rat. There is marked cellularity and no fat vacuoles are apparent. Normoblasts are preponderant. × 500.

FIG. 2. Appearance of femoral marrow 13 days after irradiation. Cellularity is diminished and fat vacuoles are abundant. Marrow depletion is almost wholly due to a reduction in the number of normoblasts. × 500.



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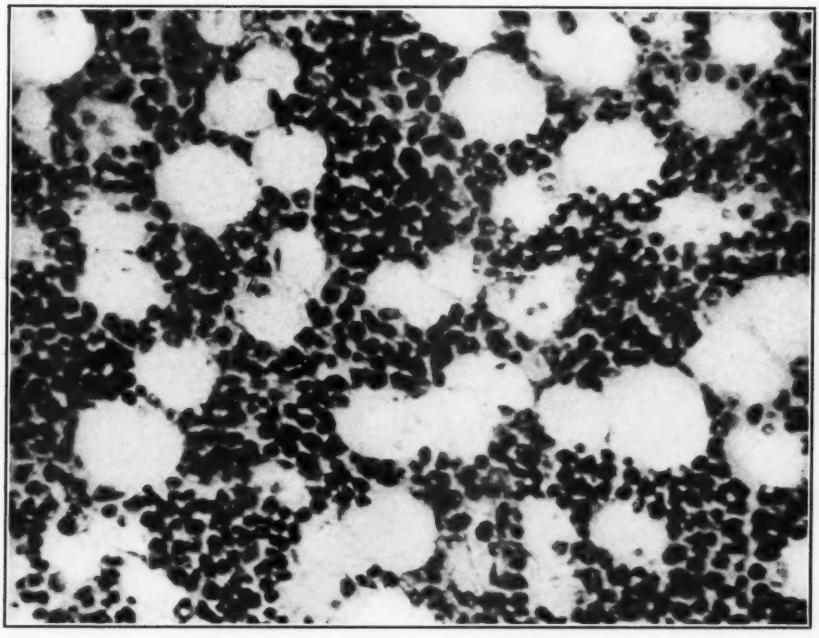

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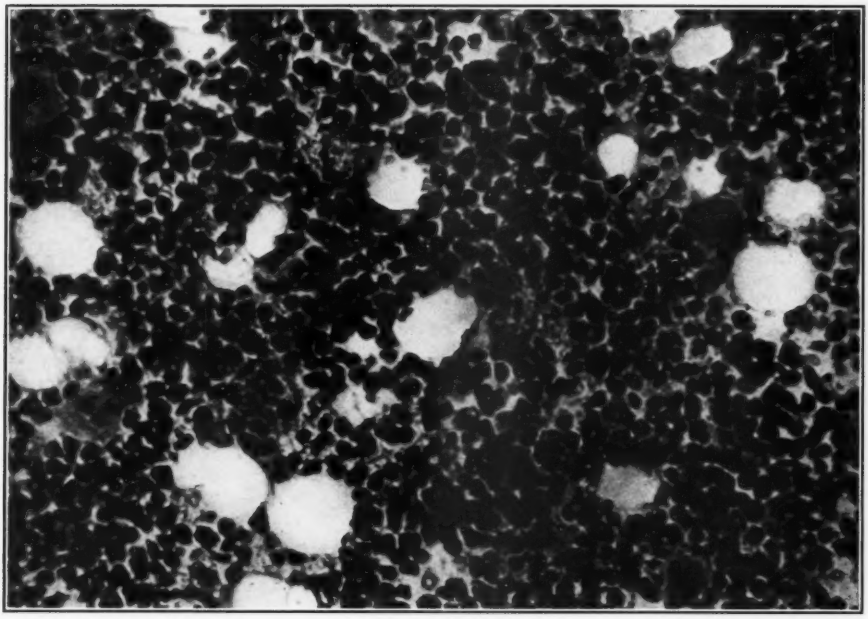
PLATE 144

FIG. 3. Apparent regeneration of marrow 28 days after irradiation. Fat content is again lessened and there is increase in cellularity. This increase is only slightly contributed to by normoblasts. × 500.

FIG. 4. Profound hypoplasia of shaft marrow 56 days after irradiation. Fat vacuoles are very numerous, cells scanty and fibrosis minimal. × 500.

FIG. 5. A section of metaphyseal marrow from the femur illustrated in Figure 4. Aplasia is marked but replacement is manifested by edematous fibrous tissue with no fat. × 500.



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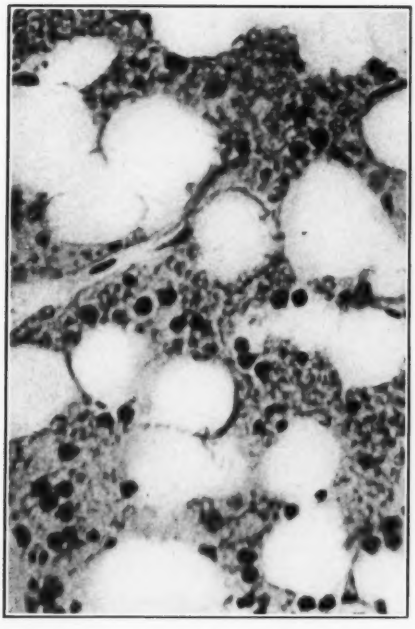

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